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## Functional analysis of root-knot nematode (*Meloidogyne javanica*) virulence genes in rice

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### Factors that influence the structure of *Citrus tristeza virus* populations

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A virus infection of a host is not a discrete, static entity, but a dynamic population whose members interact with one another and with their host to persist and spread. We have been using populations of *Citrus tristeza virus* (CTV) strains to examine this phenomenon, and have found that under constant conditions, the population structure will stabilize to an equilibrium defined by the host. This led us to ask what determinants, other than the host, can define a population, and what factors can cause a shift in population structure. Here we examined the effect of (a) inducing stress on the host through drought, high temperature, and by inducing systemic acquired resistance through the application of salicylic acid (SA), and (b) the addition of *Citrus tatter leaf virus* (CTLV) and *Citrus leaf blotch virus* (CLBV). We found that the structure of a CTV population comprised of three strains showed no significant change between treated and non-treated plants when exposed to drought or treated with SA, nor did the addition of CTLV or CLBV. Only exposure to approximate Florida summer temperatures (36°C day/25°C night) caused a change in structure, decreasing virus titer in a strain-specific and host-specific manner. Knowledge of the factors that determine population structure will aid in developing methods to manipulate populations and prevent disease.

### Virus populations associated with Maize lethal necrosis (MLN) in East Africa

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Maize lethal necrosis (MLN) has emerged as a devastating disease in East Africa in recent years. MLN is caused by co-infection of a maize-infecting potyvirus and maize chlorotic mottle virus (MCMV). Deep sequencing of MLN-associated samples collected in Kenya and Uganda indicate high conservation of MCMV sequences, whereas the associated potyvirus population is diverse and complex. Sugarcane mosaic virus (SCMV)-like sequences are abundant and variable across samples, and clustered into four groups with over 10% nucleotide sequence divergence. Sequence analyses indicate presence of other putative viruses in MLN-diseased tissue that require further characterization. These data are valuable to inform diagnostics, containment, and management of destructive maize viruses in East Africa.

### Investigating the spread of Grapevine red blotch-associated virus

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Red blotch is a recently recognized viral disease of *Vitis* spp. The causal agent, Grapevine red blotch-associated virus (GRBaV) has a single-stranded, circular DNA genome (3,206 nt), and is a proposed member of the family *Geminiviridae*. GRBaV has been detected in all major grape-growing regions of the United States and Canada, likely as a result of the dissemination of infected propagation and planting material. Of major concern to the grape industry is whether GRBaV is transmitted in vineyards. While there is no indication of spread in most areas where GRBaV has been detected, within-vineyard transmission is documented in California, likely due to insect vectors. To identify insect vectors of GRBaV, a detailed census of hemipteran insects in a selected California vineyard was performed. Of more than 50 species screened in 2015, only four species of the families Cicadellidae, Membracidae, and Cixiidae consistently carried genetic elements of GRBaV, as shown by multiplex PCR. The ability of vector candidate species to

transmit GRBaV to healthy grapevines is being assessed in greenhouse transmission assays. Understanding the mechanism of transmission will inform proper management strategies to control the insect vector and minimize spread of GRBaV.

#### **Epidemiological insights into an emerging virus disease: *Blueberry shock virus* on cranberry**

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Berry scarring, where cranberry uprights (shoots) produce scarred, disfigured berries that turn prematurely red, is an emerging problem for growers in WI and MA. We detected *Blueberry shock virus* (BShV) on symptomatic uprights using DAS-ELISA and PCR, and initiated epidemiological studies. Similar to BShV in blueberry, symptomatic cranberry uprights 'recover' from symptoms in the following season. These recovered uprights continue to test positive for BShV and had greater incidence of BShV in seeds (71%), compared to symptomatic uprights (12%). Studies on yield in three WI marshes affected by BShV revealed that flowering is comparable in healthy, recovered, and symptomatic uprights. By the time of harvest, symptomatic uprights produced fewer marketable fruit and had lower fruit weight when compared to healthy uprights. Yield components on recovered and healthy uprights were comparable. Phylogenetic analysis of the coat protein of cranberry isolates from WI (n=12) and MA (n=5) revealed 96-100% nucleotide similarity among isolates, and isolates did not group by geography. Analysis of the protein sequence revealed that BShV on cranberry shares only 90% coat protein identity with BShV on blueberry. Small RNA sequencing and assembly (sRSA) indicates genome-level single nucleotide polymorphisms in BShV from cranberry, suggesting different strains on blueberry and cranberry. In-depth analysis of sRSA data to identify other known and unknown viruses is underway.

#### **Threat to control Canna Yellow Streak Virus; altered infectivity by associating with a Sub-viral element of novel Canna Yellow Mottle Virus**

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Canna Yellow Mottle Virus (CaYMV) poses a significant threat to the canna industry by causing unwanted virus-like symptoms to the foliage. CaYMV was first reported in 1979 and the full genome has not been reported. In order to determine the full CaYMV genome and characterize the disease, next generation sequencing was carried out using Virion DNA extracted from infected canna plants. The full CaYMV genome consisting of 6976 bp with two associated sub-viral elements (SVE), was recovered. The CaYMV genome and two sub-viral elements were cloned and sequenced using PCR primers. With initial diagnostic experiments using multiplex PCR reported in 2015, CaYMV was found to be associated with Canna Yellow Streak Virus (CaYSV). To determine the nature of this association, analysis of naturally infected canna varieties including greenhouse-grown and field-grown canna for the presence of CaYMV, CaYSV and SVEs was conducted using specific diagnostic primers for CaYMV and SVE 0036. Among six canna varieties, one variety "Australia" tested positive for only the SVE-0036 while another variety "Striped Beauty" tested positive for all three. Sap containing SVE0036, CaYSV, or CaYMV were mixed and used to inoculate bean or lupine plants. Infectivity of CaYSV with the presence of SVE0036 altered in bean and lupine plants. These data suggest that it is important to consider the presence of SVE0036 when testing for CaYSV infectivity.

#### **Trivapro™ fungicide, a new fungicide for broad spectrum control of diseases of corn, soybeans, and wheat**

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Trivapro™ fungicide was registered late in 2015, and is now commercially available for use on corn, soybeans, and wheat. This fungicide contains three broad spectrum ingredients (a strobilurin, triazole, and an SDHI) that all have different modes of action. The SDHI component, Solatenol™ is an extremely potent active ingredient that provides long residual control of many important diseases. This combination of active ingredients provides both preventive and curative disease control. Trivapro is particularly effective against many of the rust pathogens. Results from corn and wheat trials in the US in 2015 demonstrated the grower benefits of longer lasting control. Research has also shown some crop enhancement benefits.

#### **Evaluation of fungicides for management of Fusarium wilt of watermelon**

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Fusarium wilt of watermelon, caused by *Fusarium oxysporum* f. sp. *niveum*, is an economically important disease in the United States and worldwide. It has traditionally been managed with long crop rotations and resistant varieties. However, the development and increased prevalence of new pathogen races has reduced the number of available resistant cultivars. In light of this, new chemical control options would greatly assist growers in controlling Fusarium wilt of watermelon. This study evaluates new and existing chemical control options for their potential in limiting *F. oxysporum* growth in the lab and managing disease in the field. Fungicide efficacy *in vitro* using a fungicide-amended media assay with 10 single formulation products at four concentrations revealed that prothioconazole and ADEPIDYN™ fungicide were among the best performing fungicides. Sensitivity to these fungicides was determined for 100 isolates of *Fusarium oxysporum*. Field-testing of these two products at two concentrations and application methods indicated that both products significantly reduced disease incidence, but a drench treatment followed by a foliar spray treatment 14 days post transplant was superior in reducing Fusarium wilt incidence in comparison to either product used in only a drench treatment. These two products could effectively complement existing strategies in managing Fusarium wilt of watermelon.

#### **Pimaricin: A new post-harvest fungicide for selected citrus, stone, and pome fruits**

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Pimaricin (natamycin) is a polyene macrolide antimycotic produced by *Streptomyces natalensis*. It has been demonstrated to block fungal growth by binding to ergosterol within cell membranes. In use as a food preservative for many years, no resistance to pimaricin has ever been observed in *Penicillium* spp. populations. Pimaricin has potential to be an effective naturally derived fungicide for postharvest management of fruit decays when used by itself (e.g., stone fruits) or in mixtures with conventional fungicides (e.g., citrus and pome fruits) for improved efficacy and as an anti-resistance strategy. We tested the *in vitro* toxicity of pimaricin against selected postharvest pathogens of fruit crops. Mean EC<sub>50</sub> values for inhibition of mycelial growth of isolates of *Alternaria alternata*, *Botrytis cinerea*, *Geotrichum citri-aurantii*, *P. digitatum*, and *P. expansum* were 0.92 mg/L, 0.79 mg/L, 3.20 mg/L, 1.47 mg/L, and 1.14 mg/L, respectively. Treatments of inoculated citrus (*G. citri-aurantii*, *P. digitatum*), stone fruits (*B. cinerea*, *Monilinia fructicola*, *Rhizopus stolonifer*), and pome fruits (*A. alternata*, *B. cinerea*, *P. expansum*, *Mucor piriformis*) with 1000 mg/L pimaricin were mostly effective with a reduction in decay incidence by 65 to 95% as compared to the control. However, reduced efficacy or treatment failures were observed in some pathosystems (e.g., *P. expansum* on pears). The mechanism for this unique selective performance is currently being investigated.

#### **Evaluation of new fungicides for management of Phytophthora root rot of citrus**

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Phytophthora root rot (PRR), caused by *Phytophthora* spp., is an important disease of citrus in California. For its chemical control, only mefenoxam and potassium phosphite are currently registered. Mefenoxam resistance is widespread, and overuse of phosphonates may lead to resistance in *Phytophthora*

spp. Thus, we evaluated the efficacy of new chemicals belonging to different modes of action (i.e., ethaboxam, fluopicolide, mandipropamid, and oxathiapiprolin) against PRR. Navel orange trees on Carrizo rootstock were inoculated with *P. parasitica* at planting. Applications with 12 fungicide treatments were made five weeks after planting, in spring and fall 2014, and in spring 2015. Feeder roots and adjacent soil were collected before or after application. PRR incidence and pathogen populations were determined by plating root segments and soil suspensions, respectively, on a selective medium. All of the new fungicides reduced PRR incidence and soil populations significantly to very low levels as compared to the control starting with the first application. Mefenoxam was only effective when a high label rate was used in the fourth application. Most of the new fungicides also increased tree canopy size and trunk diameter compared to the control. Greenhouse studies confirmed the efficacy of these new fungicides. Once registered, these new compounds will provide highly effective treatment options and resistance management strategies in the control of PRR of citrus.

#### **Improving genomic resources for the detection of *Macrophomina phaseolina* infection of strawberry**

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*Macrophomina phaseolina* is the causal agent of charcoal rot, which is characterized by wilting, stunting, and crown discoloration. It has had an increasingly devastating impact on strawberry production in California as treatment methods have shifted from broadcast pre-plant fumigation with methyl bromide+chloropicrin to individual bed treatment with alternative fumigants. Hundreds of *M. phaseolina* isolates were collected from infected hosts in multiple countries and states, including many from California. An SSR analysis revealed that, with few exceptions, the strawberry isolates were a single genotype (isolates from other hosts were not pathogenic on strawberry). To quickly identify the strawberry genotype, genotype-specific primers were developed with the end goal of implementing a diagnostic assay from soil prior to planting. These primers were designed by sequencing multiple isolates and using comparative genomics to identify genotype-specific loci. However, the complexity of the differences in the genomes of strawberry and non-strawberry isolates cannot be fully resolved with this approach. Therefore, PacBio sequencing was used to assemble a strawberry isolate reference genome. The draft assembly has an N50 of 3.3 Mb with 143 contigs and ~150x genome coverage. Ongoing work to improve and annotate this assembly is being done so that it can be used to identify additional strawberry-specific loci and to understand host specificity through comparative genomics.

#### **Identifying sources of inoculum and timing of tissue infection by fungal pathogens associated with winterberry fruit rot**

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Winterberry is a valuable deciduous ornamental plant carrying brightly colored berries during the winter that is popular as a cut green for use in holiday decorations. In the past 3-4 years, a fruit rot has been challenging winterberry growers in the midwestern and eastern U.S., in some cases leading to complete crop loss. In this study, we monitored winterberry 'Bonfire' and 'Sparkleberry' throughout the 2015 growing cycle in two Ohio nurseries to identify sources of inoculum and assess periods of tissue infection. Twigs and mummified fruit were collected during plant dormancy to determine potential sources of primary inoculum. Flowers, leaves, and berries were collected weekly from June to December to record leaf spot and fruit rot incidence and severity, as well as pathogen recovery. Spore traps were used during the growing season to monitor spore abundance in the orchards. Based on the isolation results as well as counts from the spore traps, it appears that the disease may be due to a fungal complex, including species of *Alternaria*, *Cladosporium*, *Colletotrichum*, *Epicoccum*, *Fusarium*, *Phoma*, and *Phomopsis*. The major source of primary inoculum appears to be the mummified fruit. Leaf spots may serve as a source of secondary inoculum. A significant increase in the number of fungi recovered at bloom indicates that flowers could serve as a point of entry for fruit infections. The ultimate goal of this study is to provide growers with appropriate management strategies.

#### **Occurrence of foliar diseases of watermelon on commercial farms in South Carolina in 2015**

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The main foliar diseases of watermelon in the southeastern U.S. are anthracnose, downy mildew (DM), gummy stem blight (GSB) and powdery mildew (PM). A survey was conducted in South Carolina in 2015 to obtain current data on the occurrence and distribution of these diseases. A stratified two-stage cluster sampling design was used, with 6 pre-selected counties as strata, 17 randomly selected growers as the first cluster and 21 randomly selected fields as the second cluster. Diseased leaves (100 per field) were collected along 4 transects encompassing 2500 m<sup>2</sup> with 5 equidistant sampling points along each transect. All leaves were examined microscopically, and all pathogens present were recorded. Data were analyzed with SAS survey procedures for means and proportions, logistic regression and frequency analysis. The estimated statewide probability of occurrence (and range across counties) was 0.48 (0.32-0.78) for GSB, 0.21 (0.00-1.00) for PM, 0.15 (0.00-0.49) for DM and 0.06 (0.00-0.36) for *Cercospora* leaf spot (CLS). No anthracnose was found. Field size significantly influenced the probability of pathogen occurrence; with increasing field size, the probabilities decreased. More than 30% of leaves had two or more pathogens. Associations between pathogen pairs were analyzed. The only significant interaction was that CLS was more likely to occur when GSB was present on the leaf. This study will help to match fungicide recommendations to the most frequent diseases.

#### **Worldwide distribution of the sweet potato strains of *Ceratocystis fimbriata***

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*Ceratocystis fimbriata* is a complex of species with a wide host range and worldwide geographical distribution. However, a sweet potato strain appears to be host specialized to cause black rot of storage roots. Isolates from black rotted roots of sweet potato (*Ipomoea batatas*) in China, Japan, Indonesia, Papua New Guinea, New Zealand and the USA had identical ITS rDNA sequences and only minor variation in 14 microsatellite loci (only two alleles each for two loci, and the other loci monomorphic). Three *Ipomoea* microsatellite genotypes (C1, C2 and C3) were found among the 12 isolates from sweet potato in China, two genotypes were found in the three isolates from Japan, and the other 17 isolates had identical microsatellite markers. The DNA sequences of the mating type genes of sweet potato isolates were most similar to those of isolates of *C. fimbriata* from various host plants in Ecuador, a center of diversity for sweet potato. *Ceratocystis fimbriata* on sweet potato was first described in 1890 in eastern USA, where black rot has been known for 150 years. It is hypothesized that the fungus was introduced to Japan on sweet potato storage roots and introduced from there to China in the 1930s.

#### **Fungi associated with cankers and wounds of cultivated and wild olives in the Western Cape, South Africa**

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Olive (*Olea europaea* subsp. *europaea*) production in South Africa is centred in the Western Cape Province, where viticulture is the main agricultural enterprise. Wild olive trees (*Olea europaea* subsp. *cuspidata*) also occur naturally in this region. Trunk pathogens of grapevines such as *Eutypa lata*, *Neofusicoccum australe*, *Pleurostoma richardsiae*, and species of *Phaeoacremonium* have been associated with olive trunk diseases in California, Italy and Spain, but the incidence of these on South African olive trees are unknown. The aim of this study was to identify fungi associated with cankers and wounds of cultivated and wild olives in the Western Cape Province of South Africa. Isolations from 125 olive and 27 wild olive trees sampled in nine

districts yielded a fungal collection dominated by an undescribed *Pseudophaeomoniella* species. This species has been identified from 54 olive (43%) and 15 wild olive trees (47%) in eight districts. Other fungi, including known olive trunk pathogens, occurred at incidences of 13% or less. This stands in contrast to high incidences of the Botryosphaeriaceae and *Phaeoacremonium* reported on olive trees in California and Italy. The high incidence and distribution of *Pseudophaeomoniella* sp. suggests this fungus plays an important role in olive trunk diseases in South Africa.

#### **Multiple mechanisms of nematode control conferred by seed and root applications of microbial biocontrol agents, *Trichoderma* and *Bacillus***

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Nematode diseases of plants are estimated to contribute \$150B USD in annual crop losses around the world due to Cyst (*Heterodera* and *Globodera* spp) and Root knot nematode (*Meloidogyne* spp) infection in species ranging from Legumes to Solanaceae to Brassicaceae among others. Chemical and host resistance resources are available for control however a common recommended practice is rotation to nonhost crops. Biological control of nematode diseases has been shown in the literature with the main mechanism being direct antagonism. 2015 field observations of soybeans in Minnesota USA (strip plots) and Gauteng South Africa (replicated small plots) indicated significant control of Soybean cyst and Root knot nematodes using *Trichoderma* and *Bacillus* seed treatments. Subsequently, a split root greenhouse experiment was conducted to identify strain specific activities and gain insight into control mechanisms. Tomato plant root systems were split and transplanted into two pots, both infested with *M. javanica*, such that individual plants would be influenced by two pots each. One of the two pots was treated with an individual biocontrol strain or strain combination as a soil drench. Twelve treatments were tested each with six replicates. The results showed excellent control of nematodes by some strains and strain combinations, synergistic effects between strains, and multiple mechanisms of control. Mechanisms and implications for population control will be discussed.

#### **Enhanced nematicidal activity of abamectin via nanoparticle formulation**

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Abamectin (Abm) has broad nematicidal activity but its usage is limited to seed coatings due to its high affinity for soils. To overcome this limitation, Abm was infused into plant viral nanoparticles (PVN<sup>Abm</sup>) based on *Red clover necrotic mosaic virus*, a virus that's normally transmitted through the soil sans vector. An added benefit of PVN<sup>Abm</sup> formulation is the increased diffusion rate of Abm through the soil. PVN<sup>Abm</sup> was found to be stable and have low affinities for soils collected from commercial fields (planted with corn, soybeans and sweet potatoes) in North Carolina even in samples with greater than 3% organic content. PVN<sup>Abm</sup> was found to increase the duration that Abm is nematicidal by 2-4 fold in comparison to commercial formulations. In sterile and live soil greenhouse testing, PVN<sup>Abm</sup> provided a 50% reduction in galling and egg quantities per root for tomatoes challenged by root knot nematode (RKN). When applied to turf, PVN<sup>Abm</sup> is capable of moving Abm below the thatch layer and a single application was found to retain appreciable turf greening when tested on turf infested with sting nematode. Microplot field trials are under way to determine the performance of PVN<sup>Abm</sup> under different application protocols/conditions and to quantify its effectiveness in reducing RKN/soybean cyst nematode damage in row crops and sting nematode in turf.

#### **Interaction between *Fusarium* and soybean cyst nematode on soybean**

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The soybean cyst nematode (SCN), *Heterodera glycines*, and *Fusarium* spp. are soilborne organisms that constrain soybean [*Glycine max* L.] production in the United States. While *Fusarium* and SCN are known to coexist in soybean fields, our knowledge of how these organisms interact and the implications for management is limited. The objective of this study was to determine if any interaction occurs between *Fusarium* spp. and SCN on soybean under greenhouse conditions. Pre-germinated soybean seedlings of SCN-susceptible (Williams 82) and SCN-resistant (Jack) cultivars were grown in cone-tainers filled with a sand-clay soil mixture (2:1) infested with inoculum of either of the four *Fusarium* spp. (*F. proliferatum*, *F. virguliforme*, *F. sporotrichioides*, and *F. subglutinans*). A suspension of 2,000 egg/ml suspension of SCN HG type 0 were used for inoculations at planting. The experiment was set up as factorial design with five replications for each treatment (*Fusarium* alone, SCN alone, and a combination of SCN and *Fusarium*) and repeated twice. After 39 days, disease severity as percentage of lesion length on soybean roots and SCN egg counts from soybean roots were evaluated. For disease severity, significant effects of the treatment involving *Fusarium* spp. was observed on the two soybean cultivars ( $P = 0.01$ ). Our results from the interaction study between *Fusarium* spp. and SCN illustrates the need for developing targeted approaches to manage the *Fusarium*-SCN complex on soybean.

#### **Effect of Seed Treatments on Root-Knot Management for Soybean and Cotton**

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Biological control is being accepted as an alternative to chemicals methods. Experiments were conducted at Mississippi State University to determine the efficacy of potential biological control products for management of the root-knot nematode. Seed applied biological products were received from Albaugh, LLC. Cotton and soybean seeds were treated with nine and seventeen biological compounds, respectively. Seeds were planted in 500 cm<sup>3</sup> of a steam sterilized sand: soil mix 10 cm Dia. clay pots and remained for 50 days. Each test included Abamectin and ILeVo as industry standards. Seed applied biological compounds significantly reduced root-knot nematode development on cotton and soybean. Seeds treated with Abamectin, and ALB-EXP5-1+ALB-SAR1 significantly reduced juvenile development and number of eggs produced on cotton. On soybean seeds treated with Abamectin and Bionematicide+thiabendazol+ALB-GG reduced root-knot juvenile development. Abamectin and SAR+Harpin protein+Thiabendazol reduced the number of eggs that developed on soybean roots. Seed applied biological products may have potential in nematode management.

#### **Adaptation of soybean cyst nematode populations to the PI 88788 source of resistance from 2000 through 2015 in Iowa and the effects on soybean yields**

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Most soybean cultivars grown in Iowa with resistance to the soybean cyst nematode (SCN), *Heterodera glycines*, have SCN resistance genes from a breeding line named PI 88788. Each year, field experiments are conducted throughout Iowa to evaluate 60 or more SCN-resistant and three or more SCN-susceptible cultivars at up to nine locations. Data on SCN population density, SCN virulence (HG type), soybean yield, precipitation, and growing degree-days from the experiments conducted from 2000 through 2015 were compiled and analyzed to determine how these factors affected SCN reproduction and soybean yield. Reproduction of SCN was positively correlated with temperatures and negatively associated with precipitation. Reproduction of SCN on resistant soybean cultivars with resistance from either PI 88788 or another source, Peking, was positively correlated with increasing virulence on these resistance sources. Both virulence on PI 88788 and reproduction on PI 88788-derived cultivars increased from 2000 to 2015, but not so on Peking. Yields of susceptible, PI 88788-derived, and Peking-derived SCN-resistant soybean cultivars were negatively correlated with SCN reproduction. Results indicate that virulence to PI 88788 is increasing in SCN populations in Iowa, resulting in greater SCN reproduction and yield loss. This trend is problematic because almost all SCN-resistant soybean cultivars available for Iowa farmers have resistance genes from the PI 88788 breeding line.

### **Spatio-temporal analysis of rice blast incidence in China**

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Rice blast caused by *Magnaporthe oryzae* is a devastating disease worldwide. Knowledge on the spatial and temporal patterns of disease risk at regional scale is important to understand its epidemic. In this study, rice blast incidence was surveyed in the main rice production areas in China between June 10 and September 10 from 2009 to 2014. Rice blast often had two peaks each year, and was highest in 2012 among these years. For the major production areas other than north eastern China, the disease incidence was highest in the Yunnan-Guizhou plateau, followed by Sichuan Basin and southern China, and lowest in central and eastern China. Spatial autocorrelation analysis revealed a significant correlation at distances ranging from 110KM to 340KM. Results of iso cluster unsupervised classification conducted in GIS on interpolated incidence on different dates could classify rice production areas into low and high disease risk regions. Although the regions with high disease risk varied among years, some regions frequently had high disease risk, such as areas along the border of Yunnan and Guangxi, the border of Sichuan and Chongqing, the border of Fujian and Guangdong, and the junction of Jiangxi, Anhui, and Zhejiang. The results of hot spot analysis were consistent with those of cluster analysis. The spatial and temporal patterns of rice blast obtained from this study provided a reference to efficiently organize resources for better management of the disease in China.

### **Association between temperature sensitivity and virulence of *Blumeria graminis* f. sp. *tritici***

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Wheat powdery mildew caused by *Blumeria graminis* f. sp. *tritici* (*Bgt*) is a highly destructive disease in China, which is highly sensitive to meteorological factors, especially temperature. In order to clarify the co-evolution of temperature sensitivity and virulence of *Bgt*, we collected a total of 90 isolates from 5 provinces (cities), including Yunnan, Sichuan, Beijing, Henan and Shaanxi, and tested the virulence of isolates according to 32 wheat varieties or lines with known powdery mildew resistant genes and sensitivities to temperature by detached leaf segment method. The results showed that the mean ET<sub>50</sub> of tested isolates was 23.22°C, with the highest 25.15°C and lowest 19.78°C, respectively. The ET<sub>50</sub> value of 73.34% of isolates ranged from 22.0°C to 24.0°C, and 10% of isolates below 22°C, 16.67% of isolates above 24°C. The virulence gene diversity index of the 90 *Bgt* isolates was 0.2370. The highest index *H* of isolates which from Sichuan Province is 0.2212 while the lowest index *H* of isolates from Henan Province is 0.1975. The results showed that there was a significant negative correlation between temperature-sensitivity and virulence diversity of *B. graminis* f. sp. *tritici* populations.

### **Spatio-temporal aspects of rust and nutrition of coffee plants in different irrigation systems**

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The rust (*Hemileia vastatrix*) is the most important disease of coffee and the intensity depends on productivity, cropping system, plant nutrition and water availability. Thus, the objectives were to evaluate the spatio-temporal disease progress and the relation with plant nutrition and soil fertility in coffee farms irrigated by drip and center pivot, using geostatistics. The drip and pivot areas were georeferenced with 52 points in 11ha and 50 in 17ha, respectively. The disease incidence was evaluated in 60 leaves, in five plants/point at 60-days intervals, from August 2012 to May 2015, totaling 17 evaluations. It was also evaluated at each point, productivity, mineral nutrition of the plants and soil fertility annually. These data were correlated with the rust incidence. The disease progress curves were plotted and the adjustment of semivariogram models was done. Then, the data were interpolated by ordinary kriging and the maps of disease and nutrients were constructed (ArcGIS 9.2<sup>®</sup>). The disease incidence in dripping was less than 3% over the years. In the pivot, the incidence reaching in August 2013 and from June to August 2014, between 21 and 27%. There was correlation between soil fertility and plant nutrition with rust for Ca, P and K. The spatial distribution of the disease and nutrients changed over the years. The exponential model was adjusted to coffee rust over the space. The disease gradient was observed from the foci in the kriging maps.

### **Validation and refinement of a predictive model for *Sclerotinia sclerotiorum* apothecial development in soybean fields**

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Sclerotinia stem rot (SSR), caused by *Sclerotinia sclerotiorum*, is one of the most important yield-limiting diseases of soybean worldwide and is a significant concern in the Great Lakes region of the USA. Phenologically-based fungicide applications are often inefficient, and fungicide application is sometimes unnecessary when weather conditions are not conducive for infection. Weather-based risk assessment tools are sometimes used to more accurately predict the timing of fungicide applications, however, such tools are not currently available for soybeans. In 2014, a logistic regression model using virtual weather data as inputs was developed to predict apothecial germination. The model uses 30-day averages of mean air temperature ( $r = -0.59$ ,  $P < 0.01$ ) and maximum leaf wetness ( $r = 0.35$ ,  $P < 0.01$ ) to predict the probability of apothecial presence prior to and during the soybean flowering period. In 2015, model-based fungicide applications successfully decreased disease severity and prevented yield loss in field trials. An on-site weather station was also used to validate virtual weather data. Due to inaccuracies in virtual moisture variables, higher resolution virtual weather data was combined with data collected in Wisconsin and Michigan for model refinement. The model will be incorporated into a preliminary web-based user interface to assist growers in effectively timing fungicide applications for integrated SSR management.

### **Weather-based risk assessment models for common leaf spot and black seed disease of strawberry caused by *Mycosphaerella fragariae***

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Common leaf spot of strawberry (*Mycosphaerella fragariae*) is a sporadic disease. On susceptible cultivars and under favorable weather conditions, *M. fragariae* causes black seed disease, reduced plant vigor, and reduced yield the following year. Logistic regression analysis was used to examine the relationship between weather and outbreaks of common leaf spot and black seed. Information collected at 100 location-years was used to develop the models. The response variables were fields with an average of more than 10 lesions per leaf at the beginning of flowering (10% flowering) or with black seed symptoms on more than 5% of the fruits at harvest. Nonparametric correlation and logistic regression analysis was used to identified combinations of temperature, relative humidity, and rainfall during the spring period (bud break to 10% flowering) as predictor variables. Based on model prediction accuracy, sensitivity, and specificity, only few models correctly classified fields (> 80% correct classifications). The best predictors were temperature and rain (frequency and duration). The best models were validated with an independent data set ( $n = 20$ ) and prediction accuracy was similar than the accuracy for the original data sets. Considering that management actions such as fungicide applications are not needed every year and in every field, these models could be used to identify field at risk for common leaf spot and black seed diseases.

### **Genetics behind avirulence: Identification of novel avr-genes in *Magnaporthe oryzae***

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Rice blast disease, caused by *Magnaporthe oryzae*, accounts for 10-30% of yearly rice loss which is estimated to represent enough food to feed 60 million people. The study of interactions between *M. oryzae* and rice can be used to help mitigate one of the most devastating diseases plaguing a

primary food source and knowledge of this pathosystem can be used to manage related fungal diseases. The objective of this study is to find the corresponding avirulence (Avr)-gene to the Pi-2 resistance (R)-gene in hopes of better understanding the strong relationship between *M. oryzae* and rice. In previous studies we have identified five candidate AvrPi2 genes by *Agrobacterium tumefaciens*-mediated transformation; infection assays; and TAIL-PCR with the incompatible strain KJ201. These genes have been cloned into the compatible strain Chinos. By infecting Pi-2 R rice plants with the Chinos transformants, and assessing disease, we were able to identify and characterize the most probable cognate Avr-gene. Pathogenicity assays demonstrated that two transformed lines Chinos4842 and Chinos8832 produced a similar phenotype to KJ201, with Chinos4842 showing a nearly identical phenotype match. Further, the mutants' ability to induce a hypersensitive response (HR) was assessed using 3,3'-diaminobenzidine (DAB) staining in leaf sheath assays. Of the transformed lines, Chinos4842 was the only line able to produce a HR. These results strongly suggest that MGG\_04842 is AvrPi2.

#### **Magnaporthe oryzae zinc finger effector1 exploits rice HIRA/AS1/AS2 complex to alter plant development and increase disease susceptibility**

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Rice blast, caused by *Magnaporthe oryzae*, is one of the most important rice diseases in the world. During the fungal infection, *M. oryzae* produces many effector proteins to manipulate rice cellular process and facilitate disease development. In this study, a C<sub>2</sub>H<sub>2</sub> zinc finger effector1 (MoZFE1) protein was identified from *M. oryzae* and shown to enter rice cells. Overexpression of MoZFE1 in transgenic rice (MoZFE1-OX) was found to alter rice growth and development and increase disease susceptibility. RNA-seq analysis reveals suppressions of some development- and defense-related gene expressions in the MoZFE1-OX lines. Both *in vitro* and *in vivo* biochemical analyses demonstrate that MoZFE1 directly interacts with a histone chaperon HIRA and suppresses the AS2 gene expression. HIRA/AS1/AS2 is a conserved gene-silencing complex and plays a role during plant organogenesis. Loss of function mutants (rice *hira*, *as1*, and *as2* lines created with CRISPR/Cas9) was shown to cause developmental and differentiation defects with some phenotypes similar to MoZFE1-OX lines. Our study suggests that MoZFE1 exploits rice HIRA/AS1/AS2 complex to alter host gene expression, thereby manipulating plant growth and development as well as defense response. Our findings will help elucidate the molecular mechanism of the rice-*M. oryzae* interaction and facilitate the development of new strategies to improve rice disease resistance and food security.

#### **Comparative Genomics of Two Magnaporthe oryzae Field Isolates Uncovers Novel Candidate Effector Genes**

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During the colonization process, rice blast fungus *Magnaporthe oryzae* secretes a repertoire of effectors into host tissue to overcome immunity and facilitate invasive growth. To better understand the biological functions of these complicated and diverse effectors, two field isolates Guy11 and FJ81278 were re-sequenced, and isolate-unique secreted proteins were identified through comparative genomics. When comparing with each other, 37 and 18 unique genes encoding secreted proteins were obtained in the genome of Guy11 and FJ81278, respectively. Consistent with the known effector features, the majority of these proteins are small, cysteine-rich, specifically expressed at the early stage of infectious process, and possessed presence or absence polymorphisms among different field isolates. Meanwhile, due to the different origin of Guy11 and FJ81278, the two sets of unique genes owned the diverse distribution patterns in the strains isolated from different rice varieties (*Oryza sativa* ssp. *indica* and *japonica*). Furthermore, we have identified two unique proteins could suppress BAX-triggered programmed cell death in *Nicotiana benthamiana* so far, which indicated they are novel effectors. In conclusion, our comparative genomics approach is effective to generate a list of unique genes which could be candidate effectors and identify the novel effector genes. \*To whom correspondence may be addressed. E-mail: wangzh@fafu.edu.cn

#### **Molecular dissection of a complex gene locus conferring high virulence on barley cv. Bowman in the fungal cereal pathogen Cochliobolus sativus**

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*Cochliobolus sativus* (Anamorph: *Bipolaris sorokiniana*) is a fungal pathogen causing spot blotch, common root rot, and black point in cereal crops. For spot blotch, four pathotypes (0, 1, 2 and 7) have been identified based on their differential virulence patterns on three barley genotypes (Bowman, ND5883 and NDB112), but the molecular mechanism underlying host specificity of these pathotypes is not well understood. Our previous studies indicated that isolate ND90Pr (pathotype 2) has a complex gene locus (*VHv1*) conferring high virulence on barley cv. Bowman. *VHv1* is located in an ND90Pr-specific genomic region, which contains 43 predicted genes including two for nonribosomal peptide synthetases (NRPSs). Knocking out either of the two NRPS genes led to loss of high virulence on cv. Bowman, suggesting that both NRPSs are involved in the biosynthesis of a presumably secondary metabolite virulence factor in the pathogen. We further characterized other genes adjacent and between the two NRPS genes at the *VHv1* locus and found that the two genes encoding 3-hydroxyacyl-CoA dehydrogenase and short-chain dehydrogenase/reductase (*SDR*), respectively, were also required for the high virulence of isolate ND90Pr on barley cv. Bowman.

#### **A forward genetic screen in Phomopsis longicolla provides unique insights into pathogenesis**

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*Phomopsis longicolla* (Hobbs) causes Phomopsis seed decay of soybean (*Glycine max*), and also causes lesions on soybean stems, pods, and petioles. *P. longicolla* can also exist endophytically within soybean stems, which has given rise to the hypothesis that pathogenesis occurs when the pathogen transitions from endophytic or hemibiotrophic growth to necrotrophy. In this study, a forward genetic screen was performed to elucidate genetic mechanisms underlying necrotrophy, and a RAD-seq approach was adapted to characterize genomic lesions in selected mutants. A collection of 1114 tagged, insertional mutants was created via *Agrobacterium*-mediated transformation, and mutants were evaluated individually in soybean stems for their ability to induce necrosis and form pycnidia. Six mutants induced significantly less necrosis in soybean stems than the wild-type strain, and four formed significantly fewer pycnidia. No gain-of-function mutations (increased necrosis) were observed. A modified RAD-seq protocol developed to identify the site of insertion was applied to a mutant significantly reduced in its ability to cause stem necrosis; a single copy of the disruption cassette integrated into the presumed promoter region of a cellobiohydrolase. This study describes new tools to dissect the interaction between *P. longicolla* and soybean, and provides new insight into conserved mechanisms underlying stem, pod, and seed necrosis caused by *P. longicolla*.

#### **Oomycete species richness in cacao soil assessed by massive amplicon sequencing of the cytochrome oxidase II gene**

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Oomycetes are ubiquitous in both natural ecosystems and crops, however diversity studies have focused in temperate regions. Emerging diseases together with climate change highlight the need to study oomycetes in other potential disease hotspots. Our aim was to assess Oomycete species richness and composition in soil samples from cacao farms of Ecuador by using high-throughput multiplexed Illumina sequencing of the cytochrome oxidase II gene (*cox2*). We used these nucleotide data to test the hypothesis that different oomycete species richness and composition are found between soil from cacao farms and neighboring soils, which included weeds and other cultivated crops. Eight farms were sampled covering three zones of the Coastal Region of Ecuador. Total DNA was extracted directly from collected soil samples and used for library preparation. A positive control, consisting of

gDNA from three known *Phytophthora* species, was included in the analysis. Amplicons of ~580bp were sequenced by Illumina MiSeq 2x300. Resulting reads were filtered before conducting megaBLAST analysis against a custom Oomycete sequence database. Most of the identified species were *Phytophthora* and *Pythium*. The majority of known pathogens, such as *P. palmivora*, were not restricted to cacao farms, rather they were also found in the plant communities adjacent to farms. These results suggest that wild plants and neighboring crops contribute to the ecology of Oomycete pathogens affecting cacao.

#### **Genome sequence and effector repertoire of the hop downy mildew pathogen *Pseudoperonospora humuli***

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Oomycete plant pathogens secrete effector molecules that manipulate host physiology to achieve colonization. Effector proteins have been identified in various oomycete species but little is known about the sequence, function, and evolution of effectors of downy mildew plant pathogens.

*Pseudoperonospora humuli* is the causal agent of downy mildew of hop, an economically important disease that occurs worldwide and has gained importance in recent years in the United States due to an increase of hop production. The main goal of our study was to identify and make available to the oomycete community a set of candidate pathogen effectors from *P. humuli* using transcriptomics. Identified effector candidates can be employed in high throughput functional genomics screens, commonly referred to as effectomics, to test hop germplasm for specific pathogen recognition by resistance *R* proteins. Here we report the identification of 296 sequences annotated as RXLR host-translocated effectors and the expression during *P. humuli*-hop interaction. Our findings highlight a set of *in planta* expressed RXLR candidate effectors for pathogen-assisted breeding in hop for resistance to downy mildew.

#### **What is a clone? Rethinking the ancestry of US clonal lineages of *Phytophthora infestans* using whole genome sequences**

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Identifying genetic boundaries of a clone or clonal lineage is not straightforward. Identical multilocus genotypes can arise independently from unrelated clones. Similarly, lineages derived from the same ancestral clone can accumulate variants that distinguish them. Populations of the potato and tomato late blight pathogen, *Phytophthora infestans*, are well known for emerging as novel clonal lineages. These successions of clones have historically been named within the United States as lineages US1-US24, in order of appearance. Hypothetically, these lineages can emerge through divergence from other US lineages, recombination among US lineages or as novel, independent lineages. We used 32 whole genome sequences including dominant US clonal lineages and available samples from global populations. Based on the core orthologous genes located on supercontigs 1.1 and 1.5 we observed substantial amounts of variation shared among strains within clonal lineages. We found no support for emergence of any of the US lineages from a common ancestor shared with any other US lineages. Each of the US clonal lineages fit a model where populations of new clonal lineages emerge via migration from a source population that is sexual in nature and potentially located in central Mexico or elsewhere. This work provides novel insights into patterns of emergence of clonal lineages in plant pathogen genomes and provides an evolutionary framework for definition of clones in *P. infestans*.

#### **Temperature and light effects on germination of *Peronospora effusa***

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Spinach downy mildew, caused by the biotrophic oomycete *Peronospora effusa*, is an economically important disease that is found in all spinach growing regions of the US. To effectively predict disease risk we need to understand the response of *P. effusa* to different environmental conditions. However, compared with several other downy mildew pathogens, relatively little research has been done on the optimal environmental conditions for growth and sporulation of *P. effusa*. We conducted several germination assays, exposing *P. effusa* sporangia to different temperature and lighting conditions. Between 5 and 25°C under constant darkness, germination of *P. effusa* sporangia on water agar declined following an approximately logistic decay. These results were qualitatively different from a previous study of *P. effusa* germination that found a bimodal response curve, with increased germination at lower and higher temperatures. Time course studies revealed that most sporangia germinated within the first eight hours of plating, regardless of incubation temperature. Sporangia exposed to green or blue light had significantly reduced germination when compared with those exposed to red, yellow, or no light. Light intensity and color significantly impacted germination, although the effect of color varied by light intensity. The use of these results in the development of a disease risk model will be discussed.

#### **Effect of red light on morphology of *Peronospora belbahrii* sporangia**

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Downy mildew of basil, caused by *Peronospora belbahrii*, has spread to more than 42 US states since it was first detected in Homestead, FL in 2007. Recently, our experiments showed that red light can suppress downy mildew in basil. The aim of this study was to investigate the effect of red light on biology of *P. belbahrii* sporangia. The red LED lights were hung 4 feet above the greenhouse bench and 5 feet between two adjacent LED lights. The LED in the spectrum of 625 nm wavelength illuminated at an average light intensity of 12  $\mu\text{mol}\cdot\text{m}^{-2}\cdot\text{s}^{-1}$ . The LEDs were automatically turned on from 8 pm to 8 am each day. One set of inoculated basil plants was maintained under dark conditions and another set of inoculated basil plants were placed under the exposure of red light. Fresh sporangia were harvested from the basil plants under dark conditions and under red light. A total of fifty sporangia were examined under a microscope with 40x magnification lens. The sporangia produced under dark conditions were brown in color and had oval shaped sporangia, whereas sporangia produced under red illumination were white in color and had round shaped sporangia. The change in morphology of the sporangia is an indication of a possible direct effect of red lights on *P. belbahrii* sporangia production. These results warrant further investigations to determine the mechanisms underlying changes in biology of *P. belbahrii* sporangia affected by red light.

#### **Bean common mosaic virus isolate overcomes the *bc-3* resistance allele in common bean: Virus genetic determinants defining a novel pathogenicity group**

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*Bean common mosaic virus* (BCMV) exists as a complex of strains that can be distinguished biologically, on a set of bean differentials, into pathogenicity groups (PG) numbered I to VII. Resistance against BCMV in *Phaseolus vulgaris* is governed by six recessive resistance alleles at four loci. One of these alleles, *bc-3*, is able to protect *P. vulgaris* against all BCMV strains and against other potyviruses; *bc-3* was identified as the *elF4E* allele carrying mutated eukaryotic translation initiation factor gene. We characterized a novel BCMV isolate, 1755a, that was able to overcome *bc-2* and *bc-3* alleles in common bean. Hence, it displayed a novel pattern of interactions with resistance genes in *P. vulgaris*, and was assigned to a new pathogroup, PG-VIII. The IVT7214 cultivar supporting the replication of BCMV-1755a was found to have the intact homozygous *bc-3* CAPS marker. The complete genome of the 1755a isolate was sequenced and found to be a recombinant between the BCMV strain NL1 (PG-I) and an unknown BCMV strain. Analysis of the recombination patterns in the genomes of NL1 and US1 (PG-I), NY15P (PG-V), US10 and RU1-OR (PG-VII), and 1755a (PG-VIII),



indicated that P1/HC-Pro cistrons of BCMV strains may interact with most resistance genes. This is the first report of a BCMV isolate able to overcome the *bc-3* resistance in common bean.

#### **Determination and characterization of a symptom determinant of Grapevine fanleaf virus**

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*Grapevine fanleaf virus* (GFLV) -the causative agent of fanleaf degeneration- is one of the most devastating viral diseases of grapevines. GFLV is a bipartite (+) sense RNA nepovirus and is transmitted by the ectoparasitic nematode *Xiphinema index*. The five proteins encoded by RNA1 are associated with replication and protein maturation, while the three proteins encoded by RNA2 are involved in replication, cell-to-cell movement, and genome encapsidation. Infected vines exhibit a variety of symptoms, including shortened internodes, fan-like (deeply lobed) leaves, mosaic, and vein yellowing. Infected vines have decreased fruit yield and quality, and also a shortened productive lifespan. Little is known about the host-pathogen interactions leading to symptom development. Recent work with chimeras of symptomatic and asymptomatic strains of GFLV (strains GHu and F13, respectively) in the plant model species *Nicotiana benthamiana* has shown that symptom development in this host species is dependent on a 408-nucleotide sequence at the 3' end of RNA1 of GFLV-GHu. A reverse genetics approach was used to characterize the symptom determinant by using agroinoculation to establish infection with a series of recombinant GFLV genomes *in planta* and thus determine the minimal GFLV-GHu sequence necessary for symptom development in *N. benthamiana*. A better understanding of GFLV's biology will inform the mechanism by which the virus causes symptoms in its hosts.

#### **Understanding superinfection exclusion by complex populations of Citrus tristeza virus**

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Superinfection exclusion (SIE) is a phenomenon in which a primary viral infection restricts a secondary infection with the same or closely related virus. For plant viruses, SIE, referred to as cross-protection, was implemented as an agricultural practice to protect crops against aggressive viruses by pre-immunizing plants with mild virus isolates. Recently we showed that SIE by *Citrus tristeza virus* (CTV), an economically important virus of citrus, occurs only between isolates of the same strain, but not between isolates of different strains. This work, however, was done using "pure" isolates of CTV, each containing a single virus strain, while most citrus trees in the field harbor complex populations made up of different CTV strains. The objective of this study was to assess how SIE works in plants simultaneously pre-infected with several CTV strains. Co-inoculation of citrus trees with variants of T36, T68 and/or T30 strains demonstrated that multi-strain populations equilibrate soon after establishment of infection, show uniform intra-host distribution and maintain the established equilibrium over time. SIE experiments showed that exclusion of a secondary infection by a CTV variant was triggered by the presence of another variant of the same strain in the primary population, which was in the agreement with our previous observations. The same rule appeared to be in effect when SIE by mixed virus populations was tested in a series of different citrus varieties.

#### **Potato virus Y strains co-localize and compete in single epidermal leaf cells**

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In recent years, recombinant strains of *Potato virus Y* (PVY) have become predominant in U.S. potato crops; whereas the parental PVY strains have been disappearing. Often multiple PVY strains are found in the same field and occasionally within the same plant, but little is known about how they interact or compete within the plant. Here, we examine the spatial and temporal dynamics of PVY strain infection at the cellular and tissue levels in different hosts. Immunofluorescence and confocal microscopy was used to localize mixtures of PVY strains (PVY<sup>O</sup> and PVY<sup>NTN</sup>) in epidermal leaf cells of 'Samsun NN' tobacco or 'Red Maria' potato. We observed that both strains systemically infect tobacco and are co-localized in cells of all leaves; however their relative prevalence changes over time. Early in infection when mosaic symptoms are observed, PVY<sup>O</sup> predominates the infection although PVY<sup>NTN</sup> is present in some cells. As infection progresses and veinal necrosis is observed, PVY<sup>NTN</sup> becomes the prevalent virus in those tissues outcompeting PVY<sup>O</sup>. Co-localization of PVY<sup>O</sup> and PVY<sup>NTN</sup> was also found in epidermal cells of potato leaves with almost all cells being infected with both viruses. Co-localization of virus strains in single cells is not a widely used approach for understanding interactions among them. The apparent non-antagonistic interaction among PVY strains could explain the multitude of emerging recombinant PVY strains discovered in potato in recent years.

#### **Ethylene inhibition can prevent aphid attraction and Potato Virus Y spread**

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Vector-borne pathogens influence host characteristics relevant to host-vector contact, increasing pathogen transmission and survival. Potato Virus Y (PVY) is vector-borne and considered one of the most damaging viruses for potato worldwide. Previous studies have demonstrated aphid vectors have higher fecundity and preferentially settle on PVY-infected potatoes compared to controls. Here we demonstrate PVY infection increases ethylene production and we show that ethylene production is required for increased vector attraction to infected plants. Further, we demonstrate pathogen spread can be prevented by temporarily inhibiting ethylene signaling. Virus-induced changes in ethylene responses may mediate vector-plant interactions more broadly and thus represent a conserved mechanism for increasing transmission by insect vectors.

#### **Precision diagnostics and next-generation decision support systems**

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The UK wheat crop is valued at £2 billion p.a., approximately 12% of which is lost to disease. Septoria leaf blotch (*Zymoseptoria tritici*) is the most prevalent and fungicide sprays estimated at £82million p.a. (GFK-Kynetec 2013) are used for control. Resistance is a significant problem, caused by continued overuse of chemicals with similar modes of action. Better control requires more precision in terms of spray timing as well as more careful selection of fungicides. Linking this information with risk prediction by use of disease forecasting systems is also critical. We have been developing an in-field, self-reporting, automated spore detection system, to monitor ingress of inoculum into fields. Based on cyclone air sampling technology linked to isothermal DNA amplification (LAMP) the system will sample and test air within crops, transferring data on inoculum movement wirelessly to the laboratory. In addition to inoculum, identifying resistant genotypes of a pathogen is critical to enable effective spray decisions to be made. Work has started on the development of LAMP assays to discriminate the SNPs (single nucleotide polymorphisms) responsible for azole resistance in the CYP51 gene of Septoria. The assays will be deployed on the handheld Genie III instrument, enabling decisions to be made rapidly in the field. Ultimately the two developments will converge, telling us not just when we should spray crops but also what active chemistry will be most effective.

#### **Next generation sequencing evaluation for detection of potential threats or emerging forest pest, entry point from spore and insect traps**

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International trade promotes the spread of exotic plant pathogens (fungi, oomycetes) that can act as entry points. Their spores that spread by wind, rain and through vectors (insects) can cause the introduction of exotic diseases responsible for the devastation of various plant species. Since it is difficult to trap and conduct investigations to these organisms, new technologies such as qPCR and metagenomics therefore can now be an option to detect without having to grow them. The DNA is extracted for metagenomics of spore trapping filters and insect traps conservation liquid collected in 2013-2014 and then sequenced with the Ion Torrent platform using fusion primers which we designed in order to three multiplex genic regions (350-400bp amplicons from ribosomal DNA region ITS1 for fungi and oomycetes and the mitochondrial DNA region ATP9-NAD9 for *Phytophthora* sp) and then deciphered sequences. Bioinformatic analyzes pipelines for analysis of millions of sequences are also made and compared with qPCR testing for multiple targets. Comparing the Next Generation Sequencing consistent qPCR for the detection of forest pathogens species and allowed to find several species related targets of *Phytophthora* and related Fungi. Based on the results obtained, it was possible to identify potential sources of entry, get access to contents abundancy, biodiversity and phylogeny in order and so accelerate identification process of current risk to these species for biosurveillance.

#### **An inexpensive visual PCR method for field detection of *Candidatus Liberibacter asiaticus* associated with citrus Huanglongbing**

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Huanglongbing (HLB) is threatening citrus cultivation in California and Texas. Testing for Liberibacter (Las, the HLB pathogen) by qPCR is labor-intensive and requires expensive instrumentation. The key to HLB management is large scale testing by many interested personnel. Development of on-site testing methods will increase the chance of pathogen detection by growers, homeowners and extension agents and enables timely implementation of disease management strategies. We describe an easy method to test psyllids (HLB vectors) for the presence of the Las pathogen. The initial equipment needed includes a heating block, table top centrifuge, tubes and reaction reagents. The cost for all of the equipment needed for testing by this method will be under \$300. Psyllids are captured, stored in ethanol, air-dried, and DNA extracted by heating at 80°C in extraction buffer. Pathogen DNA is amplified using a loop-mediated amplification method at ambient temperatures (65°C) for 30 minutes. Samples positive for Las will appear yellow after the reaction and negative samples will remain pink. The whole reaction can be performed within an hour in a farmhouse by non-scientific personnel with very little training. Availability of methods for large scale testing will facilitate better disease management.

#### **Comparison of two deep sequencing methods for the detection of viruses in sweet potato**

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Current methods used to detect viruses from imported accessions in plant germplasm quarantine programs face multiple challenges due to many pathogen targets, mixed infections and high genetic diversity of some pathogens. In this study, deep sequencing of small interfering RNAs (siRNAs) and RNAs (HiSeq) were compared using a sweet potato plant infected with five RNA viruses [*Sweet potato chlorotic stunt virus*, *Sweet potato feathery mottle virus*, *Sweet potato virus C*, *Sweet potato virus G*, *Sweet potato virus 2*] and two DNA viruses [*Sweet potato leaf curl virus* and *Sweet potato symptomless 1*]. Several bioinformatic approaches were used to resolve the viral genes and detect viruses and their quaspecies. Direct mapping of both read types against a crop-specific virus database resulted in rapid detection of the seven known viruses, whereas the blast search of assembled contigs from the HiSeq reads had low call on the DNA viruses. However, the genomic sequences of the viruses, especially the RNA viruses, were characterized to a high level of detail when analyzing the contigs from the HiSeq reads, enabling the detection of quaspecies. Deep sequencing is a powerful technique that can facilitate the detection and characterization of numerous pathogens in a single test, but both the sequencing strategy and data analysis aspects require careful consideration.

#### **Fluorogenic Detection of Plant Viruses by Helicase Dependent Amplification with Self-Quenched Primers**

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Thermophilic helicase dependent amplification (tHDA) is an isothermal DNA amplification that does not require temperature cycling. A study of primers sharing similar thermodynamics, but different GC content in their targeted amplification products, was made to assess fluorescence using self-quenched primers (SqP) reacting in tHDA standard temperature and chemistry. Model viruses used were *Rose rosette virus* (RRV), *High plains wheat mosaic virus* (HPWMV) formerly High plains virus (HPV) and *Hosta virus X* (HVX). RT-PCR was used as the reference method. Temperature gradients (60 to 70°C) were performed to each set of primers. tHDA reactions with SqP were optimized assessing combinations of two primer concentrations (0.15 and 0.2 µM) with two MgSO<sub>4</sub> concentrations (4 and 4.5 mM). The sensitivity of each primer set was determined by quantitative tHDA (qtHDA) in real time. Subsequently the products amplified by qtHDA with SqP were visualized in 2% agarose gel electrophoresis. Detection limits using a plasmid DNA carrying the target sequences for RRV and HPWMV are 0.0001 ng. There was no amplification of the HVX targeted region due to its putative high GC content. This study explored primer design criteria for tHDA with SqP for sensitive detection of plant viruses. The use of SqP reduces the cost of qPCR and qtHDA and has acceptable levels of sensitivity. tHDA with SqP bring new possibilities for field deployment primer design in biosecurity and microbial forensics.

#### **Long-term rotation history and previous crop effects on corn seedling health**

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Diverse rotations provide benefits to agroecosystems through changes in the soil environment. A long term experiment was established to study four different four-year rotation sequences in which the crop phase prior to corn was sampled. Soils from rotations ending with soybean, sunflower, corn and pea were tested for effects on subsequent corn in the greenhouse. Corn was grown in a soil mix containing 12% of rotation soil either infested with corn root worms (CRW) or inoculated with *Fusarium graminearum*. Seedlings were scored for root damage and shoot vigor, rhizosphere samples collected for microbiome analyses and remaining roots scanned for root measurements. Seedlings grown in soils from rotations ending with pea and sunflower had greater vigor regardless of CRW infestation. Seedlings grown in non-inoculated soils from pea rotation had shorter roots but greater root volume and diameter. Differences in germination time and vigor were stronger between *Fusarium*-inoculated and non-inoculated soils originating from pea rotations, compared to other rotations. The response to the rotation ending in pea is consistent with field observations where corn grown after pea requires less fertilizer. Analyses of the rhizosphere microbiome and seedling nutrient content, together with soil physico-chemical characteristics will provide insight on the mechanisms through which corn respond positively to rotation sequences incorporating pea.

#### **Evidence for sexual reproduction in *Fusicladium effusum***

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*Fusicladium effusum* is the causal agent of pecan scab, the most prevalent and often catastrophic disease of pecan in the southeastern USA. Despite earlier efforts to determine a sexual stage, reproduction in *F. effusum* has been observed only by asexually produced conidia. However, the degree and distribution of genetic diversity observed within and among populations of *F. effusum* are typical of a sexually reproducing fungus. The mating type locus was identified in a draft genome of *F. effusum* and was amplified and sequenced in 14 isolates collected from different geographic locations and

cultivars, revealing that 50% of the samples contained the *mtAA* idiomorph and are considered mating type A (*MTA*). The remaining samples contained *mtBA* and are mating type B (*MTB*). A multiplex PCR screen was developed to amplify *mtAA*, *mtBA* and *tubB*, and used to screen 1203 isolates of *F. effusum* from 13 pecan populations across the southeastern USA. Analysis of this collection revealed the frequency of the mating type idiomorphs is in a 1:1 equilibrium. Select isolates were crossed in pairwise combinations on oatmeal agar and after six months, organs typical of immature sexual fruiting bodies were observed, indicating existence of a putative sexual stage. Identification of a sexual stage will provide improved understanding of the disease epidemiology and pathogen population genetics, and potentially provide insight into novel approaches to manage this damaging disease.

#### **Ecological Impact of Signaling Chemicals on *Phytophthora erythroseptica***

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*Phytophthora erythroseptica* causes pink rot of potato, mostly with zoospores as major primary inocula. Zoosporic germination and infection of host (potato) are regulated by signaling molecules with quorum sensing mechanism. This study was to determine if the signals were species specific and if they were impacted by the environment. Exudates were obtained from high concentrations of fungi, bacteria, and oomycetes, as well as soil and roots of potato varieties. The exudates were used to treat zoospores of *P. erythroseptica*. Results showed that several soil microorganisms, including *P. capsici*, *P. cactorum*, *Pythium irregulare*, *Serratia* sp., *Delftia* sp., and *Microbacterium* sp. induced zoosporic germination of *P. erythroseptica*; *Pseudomonas* spp. and *Bacillus amyloliquefaciens* inhibited zoosporic germination; exudates of *Dickeya dadantii* and *Pectobacterium carotovorum* had no effect on zoosporic germination. Root exudate from the pink-rot-sensitive variety, 'Dark Red Norland,' induced zoosporic germination, while tolerant variety 'Snowden' did not. These results indicate that the zoosporic behavior is not only self-regulated, but also affected by other environmental organisms, such as host plants and soil microorganisms through signaling molecules. Whether same signals are shared among organisms is being investigated. This result may improve our understanding of the initiation and development of potato pink rot and contribute to breeding resistant potato cultivars.

#### **Model for the geographic origin and spread of the globally distributed pathogen *Phytophthora palmivora***

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The genetic structure and diversity of plant pathogens are shaped by their evolutionary history, including coevolutionary interactions with hosts, geographic spread, and long-distance migration events, often mediated by humans. In turn, diversity and dispersal of present populations impact the risk of disease epidemics and strategies for controlling disease. *Phytophthora palmivora* is a globally distributed oomycete that infects a broad range of cash crops and fruit trees in the tropics and subtropics, including cocoa and palms. The center of diversity of *P. palmivora* is in Southeast Asia, but it is an important and widespread pathogen in South America. Our multilocus sequence analysis showed that the centers of origin of the pathogen are located in the Philippines and Indonesia. We then tested alternative models for the regional and global movement of *P. palmivora*. We found genetic variation consistent with historical movement among Pacific Islands, likely associated with coconut. We also found evidence of a bridgehead effect in South America, in which the colonization of South America and host shift to cocoa led to further global dispersal, including gene flow back to Southeast Asia. We propose that the extensive genetic diversity in *P. palmivora* in Southeast Asia is the result of a complex history, including long-term co-evolution with native hosts, geographic isolation with migration, and re-introduction of genotypes from South America.

#### **The *in planta* regulon of the major *Ralstonia solanacearum* virulence regulator PhcA**

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PhcA, an important quorum sensing responsive virulence regulator of the bacterial wilt pathogen *Ralstonia solanacearum*. PhcA affects expression of virulence factors like extracellular polysaccharide, endoglucanase, and motility. However, the full extent of the regulon is unknown. We used RNAseq to test the hypothesis that the PhcA system enables Rs to transition from saprophytic to parasitic mode. An impressive 634 genes were differentially regulated at early stage of tomato wilt disease development in  $\Delta phcA$  compared to the wild-type strain GMI1000, with false discovery rate of less than 0.005. Both, RNA-seq data and validation experiments indicated that multiple anion and sugar transporters are expressed at higher levels in the  $\Delta phcA$ , suggesting that the pathogen uses this regulator to improve its survival at low cell densities. Increased expression of hemagglutinins and other adhesins in  $\Delta phcA$  suggest that at low population densities *R. solanacearum* cells may adhere more to plant surfaces and to each other. Scanning electron microscopy revealed that  $\Delta phcA$  cells form thick mats in tomato xylem vessels. These were strikingly different from the smaller, looser in planta aggregates of wild-type cells. Further,  $\Delta phcA$  was largely restricted near the point of infection whereas wild-type colonized tissues beyond the infection site. Taken together, the study identified new targets of PhcA regulation and shed more light on their biological roles in the disease cycle.

#### **Determination of CsrA/RsmA regulon in *Xanthomonas citri* subsp. *citri***

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In bacteria, it is poorly understood how post-transcriptional regulation mediated by RNA-binding proteins contributes to adaptation to environment changes during host infection. RsmA (Regulator of secondary metabolism) is a small RNA-binding protein that plays an important role in regulation of pathogenicity, motility, biofilm formation, endoglucanases and exopolysaccharide production in *Xanthomonas citri* subsp. *citri* (Xcc). In previous work, we showed that RsmA contributes to the pathogenicity by activating expression of genes encoding the type 3 secretion system (T3SS) and effectors in Xcc. However, the mechanistic understanding of Xcc RsmA in regulating motility, biofilm formation and production of exopolysaccharide and endoglucanases remains unclear. We have performed crosslinking and affinity purification followed by RNAseq analysis in order to determine the RsmA targets in Xcc cells in both rich medium and a T3SS-inducing medium. Our data revealed hundreds of putative binding mRNA targets in Xcc. Functional classification (Gene Ontology) of RsmA mRNA targets suggests Xcc RsmA is a global RNA-binding regulator that can positively and negatively affect the expression of genes in *Xanthomonas*.

#### **Construction of phosphodiesterase mutants in *Erwinia amylovora* to evaluate the role of cyclic di-GMP in virulence regulation**

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The second messenger molecule cyclic-di-GMP (c-di-GMP) is a key regulator of biofilm formation, motility and virulence in bacteria. Diguanylate cyclase (DGC) enzymes function in the synthesis of c-di-GMP and phosphodiesterase (PDE) enzymes are involved in the degradation of c-di-GMP. We have previously studied the role of DGCs in regulating virulence traits in the fire blight pathogen *Erwinia amylovora*. Here we demonstrate that all three predicted PDE genes (*edp* genes, for *Erwinia* diguanylate phosphodiesterase), *edpA*, *edpB* and *edpC* negatively regulate amylovoran production and biofilm formation. This was correlated with a significant increase in the volume of ooze produced and the amylovoran content of ooze in an immature-pear infection model using the single-gene deletion mutants *DedpA*, *DedpB*, and *DedpC*. We also observed a significant decrease in motility, virulence in immature pears, and systemic infection levels in apple shoots in the triple-gene deletion mutant *DedpA/edpB/edpC*. Gene expression levels of *dspE* and *hrpL*, both indicators of type III secretion in *E. amylovora*, were significantly reduced in *DedpA/edpB/edpC* compared to the wild type. Thus, c-di-GMP seems to be primarily involved in the positive regulation of amylovoran production and biofilm formation; in addition, increased levels of c-di-GMP also negatively regulate motility and type III secretion during the pathogenesis of *E. amylovora*.

### Characterization of RpfB-dependent DSF-Family Quorum Sensing Signal Turnover System in the Phytopathogen *Xanthomonas*

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Molecules of the diffusible signal factor (DSF)-family are a class of quorum sensing (QS) signals used by the phytopathogens *Xanthomonas*. RpfB has recently been reported as a fatty acyl-CoA ligase (FCL) that activates a wide range of fatty acids to their CoA esters in vitro. Through genetic and biochemistry analysis, we found that RpfB represents a naturally occurring DSF-family signal turnover system in *X. campestris* pv. *campestris* (Xcc) and *X. oryzae* pv. *oryzae* (Xoo). RpfB effectively turns over DSF-family signals DSF and BDSF in vivo. RpfB FCL enzymatic activity is required for DSF and BDSF turnover in Xcc and Xoo. The expression of rpfB is growth phase-dependent and its expression is significantly enhanced when *Xanthomonas* cells enter the stationary phase. DSF regulates rpfB expression in a concentration-dependent manner. rpfB expression is also negatively regulated by the DSF signaling components RpfC, RpfG, and Clp. Although most of our findings have suggested that the DSF-family signal turnover systems are generally consistent in Xcc and Xoo, we also identified some discrepancies in bacterial pathogenesis and virulence factor production between the rpfB mutants of Xcc and Xoo. Therefore, our findings strongly indicated the complexity of the RpfB-dependent DSF-family signal turnover system, which is likely fine-tuned to facilitate infection process in different hosts (rice versus crucifers).

### Integration of plant host-derived signals to coordinate developmental processes involved in *Pantoea stewartii* subsp. *stewartii* biofilm maturation

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Participation in complex, multicellular cooperative communities, known as biofilms, is a universal attribute of bacteria. Biofilm formation is now a major tenet of microbiology, yet we know little information about what environmental and physical cues drive biofilm formation in a natural context, such as during bacterial infection processes. Biofilms are encased in an extracellular matrix consisting primarily of exopolysaccharide (EPS), which holds these biofilms together, providing robustness to the architecture and making them difficult to clear from the host. The focus of this project is on the xylem-dwelling bacterium, *Pantoea stewartii* subsp. *stewartii* (*Pss*)/sweet corn pathosystem. We have placed our attention on the multi-component Rcs signal transduction system, an environmental sensing system found in *Pss* and other enteric bacteria. This system controls genes related to biofilm formation, namely EPS matrix production. We demonstrate that host plant-derived reactive oxygen species (ROS) are one environmental signal that modulates the Rcs system and this stimulation is dependent on the redox sensing transcription factor, OxyR. In addition, we demonstrate a link between a novel RTX-like, cytolytic protein in lysing host plant cells, releasing ROS and stimulating the Rcs system and subsequent EPS production, thus aiding in transition between the apoplasmic and xylem phases of Stewart's wilt disease.

### Epidemiology and Population Biology of Grape Downy Mildew (*Plasmopara viticola*) in Georgia

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The epidemiology of *Plasmopara viticola* has been studied extensively in temperate and Mediterranean climates, but limited information is available for the southeastern U.S. where hot and humid conditions may affect survival, disease progression, and population structure of the pathogen. Downy mildew epidemic progress was monitored in 2015 in a North Georgia *Vitis vinifera* vineyard on cultivars Merlot and Chardonnay. Disease onset was 2 months later than predicted by a mechanistic model for oospore germination and primary infection, highlighting knowledge gaps relative to epidemiological processes in Georgia conditions. Since timing of disease onset may be related to the mode of pathogen overwintering, pairings among single-sporangium isolates were conducted to determine the potential for sexual reproduction. Oospores were observed in about half of the pairings, indicating that both mating types are present. To better understand the relative contribution of sexual and asexual reproduction and to determine the population structure of the pathogen, isolates of *P. viticola* were collected from 19 grape varieties in 10 vineyards throughout Georgia. Initial genotyping of 96 isolates identified only one of the previously described five cryptic species of *P. viticola*, clade *aestivalis*. This study confirmed sexual reproduction, revealed preliminary pathogen population structure, and identified knowledge gaps regarding primary infection by *P. viticola* in Georgia vineyards.

### Changes in *Plasmopara obducens* population structure corresponded with the emergence and rapid spread of the impatiens downy mildew epidemics

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*Plasmopara obducens* is the newly emergent oomycete responsible for impatiens downy mildew disease (IDM) on cultivated *Impatiens walleriana*. Since IDM outbreaks began in 2004, the disease has reduced the \$187 million annual production of impatiens in the U.S. by 40%. In this study, we evaluated if there was a change in *P. obducens* population structure associated with the U.S. epidemic. Over 900 pre- and post-epidemic *P. obducens* samples were genotyped using 20 polymorphic microsatellite markers developed from a *de novo* genome assembly. Population structure analysis identified five populations (P1-P5). Prior to IDM epidemics, P1 dominated, existing as pure P1 or admixed with the other four genotypes (~50%) on North American native *Impatiens* species. The first epidemic IDM specimen collected on *I. walleriana* in 2004 from Tennessee showed an almost pure P1 genotype. Approximately 50% of the post-epidemic samples showed admixed genotypes, but admixture between P1 and the other four genotypes was uncommon. Genetic diversity was high for the four most abundant populations P2-P5 responsible for the IDM epidemics (0.56-0.78), but lower for P1 (0.37). These data showed that the recent emergence of IDM in the U.S. corresponded with several major shifts in *P. obducens* populations, with endemic P1 genotypes supplanted by novel genotypes. The high level of diversity and genetic structure in *P. obducens* populations may present difficulties for durable disease control.

### Emergent *Rudbeckia* downy mildew epidemics caused by *Plasmopara halstedii* are genetically distinct from the pathogen populations infecting sunflower

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The oomycete *Plasmopara halstedii* is regarded as the causal agent of downy mildew disease on 35 Asteraceae genera. The pathogen is also a significant problem of sunflower (*Helianthus annuus*) worldwide and more recently, a destructive pathogen of ornamental *Rudbeckia fulgida* (rudbeckia) in the U.S.A. As with other recent downy mildew epidemics on specialty crops, little is known about the pathogen causing modern outbreaks on rudbeckia. To determine if modern epidemics on rudbeckia are caused by the same *P. halstedii* genotypes as those historically found on native rudbeckia or to those causing disease on sunflower, we performed population genetic and phylogenetic analyses. Fifteen microsatellite markers were designed from a draft genome assembly generated from a *P. halstedii* isolate on sunflower. Phylogenetic and microsatellite analyses of 232 samples collected between 1883-2014 revealed two main groups corresponding to host origin. Isolates infecting sunflower hosts were further separated into eight subgroups with no obvious pattern by year, physiological race or geographical origin. Isolates infecting rudbeckia hosts were further separated into three subgroups, one corresponding to historical pre-epidemic specimens and two modern subgroups. Our results indicate that the emergent populations of *P. halstedii* infecting rudbeckia are a different species from those infecting sunflower and genetically distinct from pre-epidemic populations infecting native rudbeckia.

### **Pseudoperonospora cubensis on commercial and non-commercial cucurbits in North Carolina: Population structure determined by simple sequence repeats**

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The cucurbit downy mildew pathogen, *Pseudoperonospora cubensis*, is a major limiting factor in cucumber production in the United States (US). Each year, *P. cubensis* causes foliar destruction on cucumber, but it also infects approximately 60 host species in the Cucurbitaceae family. All major commercial cucurbits are hosts in the US. Many non-commercial hosts are grown on a small scale or occur as weeds and their contributions to the yearly *P. cubensis* epidemic are understudied. North Carolina (NC) has diverse cucurbit production and previous studies have found that NC has relatively high genetic diversity of *P. cubensis*. In this study, Simple Sequence Repeats (SSRs) were identified *in silico* from the *P. cubensis* predicted transcriptome. A subset of the identified markers were screened and evaluated for reliability and polymorphism. Eleven markers were selected and applied to *P. cubensis* isolates collected from six commercial and three non-commercial cucurbits across four time points and three regions in NC. Population analyses revealed that the greatest genetic differentiation occurs when isolates are grouped by host species more so than when grouped by location or time. These findings show host species are playing the most significant role in *P. cubensis* diversification in NC. The results suggest non-commercial cucurbits could be contributing to *P. cubensis* inoculum for certain hosts more so than others, thus influencing the population dynamics of the pathogen.

### **Species diversity and population structure of *Pythium* and *Globisporangium* in Long Island, NY ornamental greenhouses in 2014**

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*Globisporangium* and *Pythium* are plant pathogens that cause root rots, blackleg of cuttings, and damping off of various crops. Growth of the nursery and floriculture production industries, one of the fastest growing agricultural sectors in the United States, has contributed to the increase of the economic impact of ornamental plant diseases. Seasonal assessment of the diversity of species causing *Pythium* diseases on three ornamental greenhouses from Long Island, NY is providing information about the temporal dynamics of *Pythium* and *Globisporangium* species in these agricultural systems. DNA sequences of the ITS region were obtained to confirm the identity of 207 isolates collected seasonally from three ornamental operations in 2014. As in previous years, *G. irregulare* sensu lato was the predominant pathogen isolated (n=126). Simple sequence repeats (SSRs) were used to characterize the structure of *G. irregulare* s.l. populations. PCoA of ten SSR loci defined four groups based on genetic distances. AMOVA revealed significant genetic differentiation between greenhouse populations and between genetic lineages. The main lineage included most of the isolates present in two greenhouses, which suggest a potential common source of inoculum for those facilities.

### **ADEPIDYN™ fungicide: Cross resistance patterns in *Alternaria solani***

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ADEPIDYN™ is the first member of a new chemical group among the succinate dehydrogenase inhibitor fungicides (SDHI, FRAC Group 7), the N-methoxy-(phenyl-ethyl)-pyrazole-carboxamides. The common name for ADEPIDYN™ is pydiflumetofen. ADEPIDYN™ has high binding properties to the complex II enzymes that provide a strong fungicidal potency combined with the control of a wide range of plant pathogen species. A variety of mutations are associated with resistance to SDHI fungicides. Cross resistance patterns across SDHI fungicides are complex due to the fact that some mutations confer high levels of resistance and others do not and mutations could lead to different sensitivity shifts in different SDHIs. In *Alternaria solani* isolates, the *in vitro* sensitivity revealed incomplete cross resistance patterns between ADEPIDYN™ and boscalid, penthiopyrad, SOLATENOL™ and fluopyram. ADEPIDYN™ exhibited the highest intrinsic activity among these fungicides and across isolates with or without mutations in the sdh subunits (SdhB-H277Y, SdhB-H277R, SdhC-H134R, SdhD-D123E and SdhD-H133R). ADEPIDYN™ was able to control early blight disease development on inoculation studies done with *A. solani* isolates sensitive and with different levels of resistance to boscalid and Sdh mutations. Fluopyram was also able to control the disease using higher fungicide rates and disease control with SOLATENOL™, penthiopyrad and boscalid varied according to the presence or absence of Sdh mutations.

### **Fungicide resistance profiles of unique and clonal genotypes of gummy stem blight fungi from the southeastern United States**

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Gummy stem blight (GSB) is a devastating disease of cucurbits that is usually managed with fungicide applications. However, GSB fungi have rapidly evolved resistance to multiple classes of fungicides. To better understand the evolution and persistence of fungicide resistance in field populations, we studied resistance profiles associated with unique and clonal genotypes of GSB fungi. Nineteen *Stagonosporopsis caricae* isolates and 120 *S. citrulli* isolates collected from watermelon fields in the southeastern U.S. were genotyped with 16 SSR markers and a *cytb* marker for azoxystrobin (QoI) resistance, and phenotyped for sensitivity to tebuconazole (DMI), boscalid (SDHI), and fluopyram (SDHI). *Cyp51* and *sdhB* genes of select isolates with varying sensitivity to DMI and SDHI fungicides, respectively, were sequenced. All *S. caricae* isolates were resistant to tebuconazole and azoxystrobin, and sensitive to boscalid and fluopyram. All *S. citrulli* isolates were sensitive to tebuconazole, sensitive to fluopyram with one exception, and resistant to azoxystrobin with two exceptions. Phenotypic differences in response to boscalid were detected among *S. citrulli* isolates, but the phenotype was not associated with clonal genotype, suggesting multiple evolution events. This study provides critical information for effectively managing both species of GSB fungi present in the southeastern U.S., while providing novel insights on the evolution of resistance in plant-pathogenic fungi.

### **Survey for Quinone Outside Inhibitor Resistant *Cercospora sojina* in Virginia Soybeans**

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Quinone outside inhibitor (QoI) fungicides control a wide spectrum of fungal diseases, but this class of fungicides has a high risk of developing resistance. QoI fungicides are used in the management of frog-eye leaf spot (FLS) caused by *Cercospora sojina*. In 2014, FLS isolates from four fields in VA were tested, and QoI resistance was confirmed from two fields. In 2015, a statewide survey was conducted to estimate the prevalence of QoI resistant FLS in VA. Leaflet samples with FLS symptoms were collected from 55 soybean fields throughout the VA soybean growing region. Ten leaflets were randomly selected per sample and conidia were isolated from one lesion per leaflet. A total of 116 isolates were successfully cultured from 25 locations, and DNA was extracted from monoconidial cultures. A PCR-based assay was used to screen isolates for the G143A mutation in the cytochrome b gene that confers QoI resistance. Of the 116 isolates, 24% were positive for the G143A mutation. At the 10 locations with QoI resistant isolates, frequencies of the mutation ranged from 50 to 100%. DNA was extracted directly from leaves collected at all 55 locations, and a pyrosequencing assay was designed to quantify the frequencies of the G143A mutation within the *C. sojina* populations in each field. The survey confirms the presence of QoI resistant FLS in Virginia, so growers should use a different or mixed mode of action fungicides, plant resistant varieties, and rotate to non-host crops.

### **Fitness and competition studies of QoI resistant and sensitive *Cercospora sojina* isolates, the causal agent of frogeye leaf spot**

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Frogeye leaf spot (FLS), caused by *Cercospora sojina*, is a foliar disease affecting soybean, often managed by applications of Quinone outside inhibitor (QoI) fungicides. Because *C. sojina* isolates have developed resistance to QoIs, a better understanding of the biology and aggressiveness of isolates is important. Spore suspension inoculum prepared from QoI-resistant and -sensitive isolates were mixed in six different ratios. Greenhouse plants (cultivar Blackhawk) were treated: water (control), Quadris, or Quadris Top. Results were: 1) Disease severity increased as the proportion of QoI resistance increased, indicating that QoI-resistant isolates are more aggressive; 2) Quadris inhibited FLS caused by QoI-sensitive isolates only, and when the proportion of QoI-resistance was  $\leq 10\%$ ; 3) Quadris Top provided more effective control of FLS by reducing symptoms and delaying disease development. Fitness parameters measured were (i) mycelial growth and sporulation capacity, (ii) conidial germination and growth inhibition on fungicide amended media. No significant differences of the fitness parameters were observed between resistant and sensitive isolates, except for sporulation capacity and growth inhibition. Moreover, qPCR results showed resistant isolates dominated the population in all mixed inoculation tests. These results indicate there are differences in QoI resistant and sensitive *C. sojina* isolates in-planta that will influence effective disease management of FLS.

### **Assessing potential virulence differences between QoI-sensitive and -resistant *Cercospora sojina* isolates from Mississippi soybean**

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Frogeye leaf spot (FLS) of soybean is caused by the fungus *Cercospora sojina* Hara. Quinone outside inhibitor (QoI) fungicides have historically been the standard for FLS management in MS. However, widespread QoI resistance has been reported in the MS soybean production system. QoI resistance may be associated with a fitness cost that includes a loss of virulence. A comparison of virulence was assessed between two *C. sojina* sensitivity groups based on the presence or absence of the G143A substitution and termed QoI-sensitive or -resistant. Twenty four *C. sojina* isolates, 11 QoI-sensitive and 13 QoI-resistant, were assayed in an *in planta* virulence assessment. Soybean seedlings were inoculated with conidial suspensions of each *C. sojina* isolate. Percent FLS severity assessments were made 21 days post inoculation. QoI-sensitive and -resistant *C. sojina* isolates were pathogenic to soybean seedlings based on the production of typical FLS lesions. Disease severity significantly differed among isolates within sensitivity groups. No differences in disease severity were noted between sensitivity groups; however, a 4.5% increase in severity was noted for the QoI-resistant group. This suggests QoI-resistant isolates are equally virulent as QoI-sensitive isolates, indicating no fitness cost associated with QoI-resistance. Failure to control *C. sojina* with QoIs dictates the need for alternative management practices when high-yielding FLS-susceptible cultivars are planted in MS.

### **Characterization of Arabidopsis Defense-related Gene Homologues in Tomato and Strawberry**

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The United States is the largest strawberry producer, and second in tomato. Yield of these crops is threatened by a number of diseases. Durable measures for disease management have been difficult to develop. One strategy that is being pursued is utilizing defense-related genes from *Arabidopsis thaliana* and their homologues in other plants. The Arabidopsis Elongator complex plays an important role in plant immunity. Elongator is not specifically involved in pathogen recognition, subsequently, it may provide durable resistance. Our results show that overexpressing Arabidopsis Elongator genes in tomato and strawberry improves resistance to several bacterial and fungal pathogens. The predicted tomato and strawberry Elongator genes are highly homologous to those of Arabidopsis. Importantly, the tomato and strawberry Elongator gene homologues are able to complement the morphological and defense phenotypes in corresponding Arabidopsis Elongator mutants, indicating that they are functional. Generating transgenic plants with broad-spectrum disease resistance would be a promising alternative to conventional methods. However, recently there has been a strong anti-GMO propaganda aiming to reach the public. Since the tomato and strawberry Elongator gene homologues are functional, they could be utilized to generate tomato and strawberry plants with increased disease resistance, using alternative technologies that are not considered transgenic, such as cisgenesis and intragenesis.

### **Speeding up the discovery of novel sources of leaf rust resistance in wheat**

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Leaf rust (LR) caused by *Puccinia triticina*, is among the most important diseases of wheat (*Triticum aestivum* L.) crops globally. Deployment of cultivars incorporating genetic resistance, particularly the combination of adult plant resistance (APR) and all stage resistance (ASR), is considered to be the most effective and sustainable method to control rust pathogen. While scoring of ASR in the glasshouse is straight forward, identification of APR in the field is often challenging due to varying micro environments. To overcome this, here we report a novel screening procedure that involves application of a rapid phenotyping method performed under accelerated growth conditions (AGC; i.e. constant light and controlled temperature) and effective elimination of known APR genes using linked DNA markers. Using this approach, screening of 300 diverse wheat accessions from the N. I. Vavilov Institute of Plant Genetic Resources (VIR) identified a set of 50 wheat accessions as potential sources for new APR to LR. In the subsequent screening of the subset under field conditions, 13 accessions consistently displayed good levels of APR. Further, in the DNA marker screening of the 300 accessions, 88 were identified to carry known APR genes (*Lr34*, *Lr46* and *Lr67*). Interestingly, accessions carrying *Lr67* were mostly landraces from India and Pakistan, consistent with previous reports about the likely origin of this APR gene. This can accelerate discovery of new sources of resistance.

### **Mapping of two QTL conferring adult plant resistance to *Puccinia graminis* f. sp. *tritici* race TTKSK (Ug99) and evidence for race specificity**

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The durability of wheat stem rust (caused by *Puccinia graminis* f. sp. *tritici*) resistance is strengthened by the use of multiple broad-spectrum sources of resistance. Adult plant resistance (APR) is thought to provide partial effect, race non-specific resistance. APR was observed in the Ecuadorian bread wheat cv. Morocho Blanco (MB) in field tests at Njoro, Kenya. MB is susceptible to TTKSK (Ug99) at the seedling stage. A doubled haploid (DH) population was created from a cross between MB and the susceptible line LMPG-6 to identify loci associated with APR phenotypes. Eighty-eight DH lines were genotyped with approximately 90,000 SNPs using a custom Infinium assay from Illumina. Sixty-seven additional DH lines (MB x LMPG-6) and seventy-three recombinant inbred lines (MB x Faller) were used to verify SNPs associated with reduced levels of stem rust infection. Severity and infection type were assessed on adult plants in Njoro, Kenya from 2013-2015, Debre Zeit, Ethiopia in 2015, and in single race nurseries inoculated with races RCRSC, TPMKC, QTHJC, and QFCSC in Rosemount, USA from 2014-2015. Two QTL that reduce stem rust severity were identified on chromosome arms 2BS and 6AS in the three screening seasons in Kenya. However, in Ethiopia only 6AS was effective, and in the USA only 2BS was effective to race QFCSC. The current data indicates that a singular race and location should not be used to develop lines using APR as possible genotype x race interactions exist.

### Utilizing mapping-by-sequencing to identify the Dominant Net Form Net Blotch Resistance Gene from Barley Line CI 5791

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Net form net blotch (NFNB) caused by *Pyrenophora teres* f. *teres* is a destructive foliar disease in barley growing regions worldwide. The diverse pathogen populations and complex host-parasite interactions occurring in this pathosystem has hindered breeding efforts. A dominant NFNB resistance gene identified on chromosome 6H in the line CI5791, designated *RptCI5791*, provides resistance to diverse isolates and is tightly linked to the dominant susceptibility locus *Spt1*. CI5791 lacks *Spt1* as we were unable to amplify any allelic variants of *Spt1*. Thus, we hypothesize the high level of resistance in CI5791 is due to the dominant resistance gene *RptCI5791* coupled with the absence of the dominant susceptibility locus *Spt1*. To further identify and characterize this broad-spectrum resistance, we used a forward genetics approach by g-irradiation and identified five putative mutants CI5791- $\gamma$ 3, - $\gamma$ 6, - $\gamma$ 7, - $\gamma$ 8, and - $\gamma$ 9 that are susceptible. We are utilizing the barley SEQCAP exome capture array (Nimblegen) and Illumina sequencing to identify the gene using a mapping-by-sequencing method. Mutated gene/s that map to the centromeric region of barley chromosome 6H will represent a strong candidate *RptCI5791* gene. However, the possibility still exists that alleles of the CI5791 gene may not be present in the lines used in the exome capture, which would result in a failure to identify the gene. In this case we will continue pursuing the tried and true positional cloning approach.

### Development of molecular markers tightly linked to Potato virus Y resistance gene *Ryhc* in a diploid potato population

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In the last 15 years, *Potato virus Y* (PVY) has been the main plant pathogen limiting seed potato certification in North America. The emergence of necrotic and recombinant PVY strains that cause mild foliar symptoms has resulted in increased PVY incidence. The use of cultivars that do not express foliar symptoms has also contributed to the problem. Several PVY resistance genes have been identified in wild relatives of potato, but few have been introduced into North American cultivars. We identified a diploid *Solanum chacoense* clone (CHC 39-7) in accession 275138, carrying PVY resistance gene *Ry<sub>chc</sub>*, and used it in a cross with the susceptible, diploid *Solanum tuberosum* US-W4. A heterozygous resistant F1 progeny, XD3, was self-pollinated and the resulting population was phenotyped and used to develop molecular markers for genotyping. The phenotypic and genotypic results showed skewed segregation ratios, suggesting that a lethal factor is associated with susceptibility in the population. In addition, the development of markers that are more tightly linked to resistance in CHC 39-7 has aided in fine mapping *Ry<sub>chc</sub>* and elucidating gene organization on chromosome IX. Furthermore, XD3 was crossed with a high yielding, adapted diploid *S. tuberosum*-*S. chacoense* hybrid and several progeny were selected for their high yields and good tuber type. These breeding clones and molecular markers provide new tools for breeders to introduce PVY resistance into North American cultivars.

### Metabarcoding the soybean seed core mycobiome

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The goal of this study was to explore the soybean seed core mycobiome using next-generation sequencing. Eight seed samples, representing four different locations in the state of Kansas and two soybean genotypes, were used. Seeds were surface disinfested with a 5% bleach solution (0.525% NaOCl v/v) for 1 min and dried overnight at room temperature. DNA was extracted from each ground soybean sample. After amplification of the ITS region, using ITS1F and tagged ITS4 primers, amplicons were sequenced using the Illumina MiSeq platform. Approximately 170,000 high-quality reads were produced from all samples analyzed in this study. Sequences from each sample were then taxonomically assigned using the Fungal ITS classifier from the Ribosomal Database Project (RDP). Initial analysis showed that both non-pathogenic fungi, such as *Alternaria* sp., *Epicoccum* sp. and *Cladosporium* sp., as well as pathogens such as *Cercospora* sp., *Phomopsis* sp. and *Fusarium* sp. were observed in all locations and genotypes. To better understand the soybean seed core mycobiome and its implication for global food security, additional experiments are underway to explore the presence of seedborne fungi, especially regarding the pathogenic groups, among individual soybean seeds as well as among three major soybean seed tissues including the seed coat, cotyledons, and embryo axis.

### Soil microbiomes associated with alternative strategies to manage *Verticillium dahliae*

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*Verticillium dahliae* is a fungal plant pathogen that causes Verticillium wilt, which results in significant losses in many crops. Control of *V. dahliae* is difficult as the pathogen can survive in soil without a host for over 10 years. Fumigation is an effective control, but the most viable option, methyl bromide, is no longer used and alternatives are expensive and subject to strict local regulations. Our goal is to develop environmentally-friendly and economically-viable alternatives to manage Verticillium wilt. These include organic soil amendments such as broccoli residue and a mixture of crab and feather meal, which have demonstrated disease suppressive effects in prior research, likely mediated by the soil microbiome. We performed greenhouse studies on a Verticillium sensitive host, eggplant, on which we assessed Verticillium wilt severity, and characterized soil microbial communities using 16S Illumina sequencing. The experimental setup involved a randomized complete block design with three soil amendments and 4-5 replicates for each amendment, and was performed separately for two soil types with different inoculum levels. The experiment was repeated once. We found that soil type, time of sampling, and amendment significantly impacted microbial communities; and that a sub-set of taxa was more abundant in disease suppressive treatments than in controls. Future studies will focus on the mechanistic bases of disease suppression by soilborne microbes.

### Root and rhizosphere microbiome responses to new tomato rootstock systems

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Grafting tomato scions onto rootstocks can increase yields by providing disease resistance, stress tolerance, and increased vigor. We hypothesized that grafting affects the rhizosphere microbiome, or rhizobiome. We analyzed impacts of grafting scion BHN589 with two rootstocks BHN1028, RST-04-106 on the rhizobiome of tomato plants using rRNA gene sequences, including non-grafted and self-grafted controls. We also compared the microbial communities in roots vs. rhizosphere. In the first year, there were no observed effects of grafting or rootstock on microbial diversity in the three farm sites. Many OTUs were shared (72% in bacteria, 65% in fungi) among treatments, while some taxa were treatment-specific. Across all grafting treatments, higher diversity was found in the rhizosphere compared to the roots ( $p < 0.001$ ). Composition of the rhizobiome varied with root-rhizosphere compartment and farm site. *Proteobacteria*, *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, and *Planctomycetes* were most abundant in all treatments, with the latter three being less abundant in roots. *Pezizales*, *Cantharellales*, and *Xylariales* were enriched in roots, whereas *Pleosporales*, *Mortierellales*, and *Hypocreales* were enriched in the rhizosphere. Interestingly, community modularity was higher in the roots compared to those from the rhizosphere. Ultimately, understanding rhizobiome responses to rootstocks will support vegetable production, rhizobiome-based crop breeding, and disease management.

### Genetic determinants of bacterial adaptation to plants

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Plants tightly associate with an array of diverse bacteria, with which they share complex and fascinating interactions, including parasitism, mutualism, and commensalism. These plant-associated (PA) bacteria have evolved a large set of genes that enable them to adapt to the plant environment. The functions of these genes are in many cases poorly studied and lack sufficient mechanistic understanding to facilitate microbiome engineering. Here, we sequenced 485 genomes of bacterial isolates and single cells isolated from the root environments of Arabidopsis, poplar, and maize. We then employed a large-scale comparative genomics study to characterize PA genes, and in particular root-associated (RA) genes. This was done by comparing 18 million genes from 3902 high quality and phylogenetically diverse bacterial genomes that were classified based on their isolation site. Several genomic features are common to most PA bacteria, such as the expansion of carbohydrate metabolism regulons. Many of the PA genes are encoded within putative gene operons, some of which encode for novel complex functions. Novel PA genes include a rapidly evolving virulence factor in the *Acidovorax* genus, a phage-like secretion system, and bacterial proteins with plant-like domains. The large PA and RA gene repository and the novel sequenced RA bacterial genomes will enable engineering of beneficial interactions for improved crop productivity and agricultural sustainability.

### Unravelling the sugarcane microbiome to resolve the Yellow Canopy Syndrome in Australia

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Yellow Canopy Syndrome (YCS) is a largely undiagnosed condition impacting sugarcane crops in Queensland, Australia, causing yield losses up to 30%. Since the first observation of YCS near Cairns in 2012, the condition has spread to all major cane growing areas and is a critical issue for the industry. Key YCS symptoms differ from leaf yellowing due to drought stress, phytotoxicity, insect attack, known diseases, nutrient deficiency or natural maturing. Despite elimination of some potential causes, the causal agent of YCS remains unknown. We aim to determine the involvement of biotic interactions and soil nutritional health in YCS development. A microbiome-based approach using Illumina MiSeq and HiSeq was applied on 598 samples to reveal the complexity of organisms (bacteria, fungi, other eukaryotes, viruses) present in YCS-affected sugarcane and rhizosphere soil. Soil nutrient status and microbial enzyme activity were also determined and did not significantly differ between affected and healthy fields. Bacterial (>2500 OTUs) and fungal (>11000 OTUs) assemblages of leaves, stalks, roots and rhizosphere soils differed between asymptomatic vs. symptomatic plants, as well as some rhizosphere microbial functions. Community shifts with YCS appeared variety-specific, and to date few consistent bacterial and fungal and no viral signals associated with YCS emerged. We envision ongoing data mining may reveal potential causal agent(s) of YCS or management practices for YCS.

### Illuminating the diversity of rust fungi infecting millets

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Millets are a diverse assemblage of grains frequently grown in subsistence agriculture or as forage crops, and their popularity as nutritional alternative grains has recently increased. Millet pathogens are not well known, and modern collections of fungi associated with millets are lacking. Rust fungi are a significant group of pathogens that cause economically important diseases of three commonly grown millets: pearl millet (*Pennisetum glaucum*), foxtail millet (*Setaria italica*), and teff (*Eragrostis tef*), a millet becoming popular as a health food. 28S rDNA sequences were analyzed from over 40 herbarium specimens of rust fungi on millets, and related grasses, housed at the U.S. National Fungus Collections. Three taxa of rust fungi were observed on pearl millet, *Puccinia substriata* var. *indica*, *P. substriata* var. *penicillariae*, and *P. cenchrini*, previously unreported on this host. The foxtail millet rust pathogen, *Uromyces setariae-italicae*, showed a high degree of intraspecific genetic diversity and was present on grasses in the Paniceae subtribes Cenchrinae and Melinidinae. The teff rust pathogen, previously known as *U. eragrostidis*, is actually distinct from *U. eragrostidis* and is identified as *U. pedicellata*. Knowledge of the diseases of millets is increasingly important as they become more widely grown as human food sources and pressure to increase yields in the presence of the pathogens intensifies.

### Genetic diversity of *Colletotrichum* isolates from *Sorghum bicolor* and *S. halepense* in the Southeastern United States

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Anthraxnose caused by *Colletotrichum sublineola* is the most important disease of grain sorghum worldwide, and it is emerging as a significant production constraint for sweet sorghum grown in the Southeastern U.S. Anthracnose is also common on the wild sorghum relative johnsongrass (*S. halepense*). Marker analysis using repetitive fingerprinting probes revealed that isolates from cultivated sorghum were generally distinct from isolates from *S. halepense*. RFLP analysis based on probes against individual sequences presumed to encode effectors and secondary metabolism enzymes showed that most isolates from johnsongrass grouped separately from most isolates from cultivated sorghum. Isolates from cultivated sorghum were generally aggressive to sweet sorghum variety Sugar Drip, while isolates from *S. halepense* generally caused little or no disease. Phylogenetic trees were inferred based on isolates from both host species using portions of the DNA lyase gene, the manganese superoxide dismutase gene, and a region between the APN1 and the MAT2 genes (MATAPN) genes. These trees were found to be congruent, and to identify two distinct species, the first one associated with the cultivated *S. bicolor*, and a second, new species associated with *S. halepense*. Strong evidence was found that cross-infection of these two species does occur, and this could be expected to complicate efforts to develop and deploy resistant sweet sorghum varieties in areas where johnsongrass is common.

### Phylogenetic and population analyses of the brown root-rot pathogen (*Phellinus noxius*) highlight the existence of two distinct populations

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*Phellinus noxius* is a vastly destructive, fast-growing fungal pathogen that affects a wide range of woody hosts in pan-tropical areas, including Asia, Oceania, and Africa. This pathogen causes brown root-rot on diverse hosts, with little indication of host specificity. Pathogenic symptoms of *P. noxius*



infection can include reduced tree growth, defoliation, and branch dieback; however, *P. noxius* can survive as a saprophyte. Understanding the genetic diversity and evolutionary history of *P. noxius* populations worldwide will help assess the evolutionary origins, worldwide movement, and potential ecological differences within *P. noxius*. The objective of this study was to estimate a wide-scale genetic diversity and evolutionary history of *P. noxius* movement. A total of 158 isolates were included from Japan, Taiwan, Australia, China (Hong Kong), Malaysia, and the Pacific islands of American Samoa, Saipan, Guam, Palau, Pohnpei, and Kosrae were sequenced at four loci including ITS, LSU-rDNA, elongation factor-1 alpha, and RNA polymerase subunit II. The results show two distinct clades of *P. noxius*. Currently, sequencing data are generating by Illumina sequencing of double-digest reduced representation libraries (ddRAD). Continued analyses will assess regional population structure, gene flow among populations, diversity in recently observed populations, and potential suitable climate space for specific genotypes.

#### **Microsatellite genotyping reveals high genetic diversity in *Phytophthora infestans* from Mexico**

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Toluca valley of Mexico is considered to be the center of origin of late blight pathogen, *Phytophthora infestans*. This hypothesis is supported by populations being sexual, mating type ratios 1:1 and a high standing genetic diversity in HWI. However, the population structure from other areas of Mexico is understudied. We hypothesize that migration of *P. infestans* outside of Toluca might have resulted in admixed population structure with decreasing diversity away from Toluca. To test this hypothesis we sampled and microsatellite genotyped more than 85 isolates from Mexico in the regions of Chapingo, Juchitepec, San Geromino, Tlaxcala and Toluca. Our analysis revealed high number of multi-locus genotypes and high allelic diversity. Juchitepec samples formed two distinct clusters suggesting two differentiated populations. Tlaxcala isolates seem to be uniformly distributed possibly indicating multiple migration events and admixture. Chapingo and San Geromino samples were more similar between them. In general, all regions showed no linkage among loci suggesting presence of sexual reproduction, except for Chapingo. Contrary to our hypothesis, diversity in other populations was even higher or comparable to Toluca. These results indicate that the center of origin is larger than previously thought. Our work provides a more refined understanding of the population structure of *P. infestans* in areas near Toluca where the pathogen has been well studied.

#### **Characterization of isolates of *Phytophthora capsici* from the mid-Atlantic region, U.S.**

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The heterothallic oomycete *Phytophthora capsici* causes disease under warm (25-28°C), wet conditions. This pathogen causes pod rot in lima bean (*Phaseolus lunatus* L.), an emerging disease in the mid-Atlantic region which reduces yields and limits production. In order to better understand sources of inoculum, isolates were collected from infected commercial plant samples, 13 irrigation water sources, potential weed hosts, and soil sediments in Delaware. Water filtration, baiting with fruits, or baiting with *Rhododendron* leaves, were used in an attempt to extract *P. capsici* from irrigation water. Species other than *P. capsici* were recovered in abundance from irrigation water, weed hosts, and soil sediments. A total of 38 isolates were recovered from symptomatic lima bean, pepper, pumpkin, and watermelon samples and positively identified as *P. capsici* both morphologically and molecularly. Isolates derived from single zoospores were characterized for mating type, mefenoxam sensitivity and virulence on eight commercial lima bean cultivars. The A2 mating type was prevalent among the collected isolates (78.9%) and seven isolates with resistance to mefenoxam were identified. All isolates were virulent on the eight lima bean cultivars tested. Two SSR markers were identified that co-segregate with mefenoxam sensitivity, with over 80% accuracy when used together, that could have potential use in disease management efforts.

#### **Epidemiology and management of bacterial leaf spot caused by novel *Pseudomonas syringae* strains on watermelon in Florida**

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Major disease outbreaks occurring on watermelon and squash caused by *Pseudomonas syringae* were widespread in Florida and Georgia during recent years. This was recognized as a new disease in the region, and affected approximately 6,500 acres of watermelon from Florida in 2013. Field trials were conducted during the 2014 and 2015 spring seasons in Quincy and Citra, FL to examine environmental conditions which may be conducive for disease progression, and to assess the efficacy of foliar application of copper with or without Ethylene bis-dithiocarbamate (ManKocide/Kocide 3000) and foliar/drip application of plant activator (Acibenzolar S-Methyl; Actigard) in managing both transplant and field level introductions of the pathogen. Pearson's correlation coefficient indicated that disease severity from the four trials was significantly correlated with average weekly temperature and total weekly rainfall in greenhouse inoculated (-0.67,  $p < 0.0006$ ; 0.51,  $p < 0.01$ ) and field inoculated plots (-0.72,  $p < 0.001$ ; 0.58,  $p < 0.01$ ) respectively. ManKocide and Actigard foliar applications were found to significantly reduce disease development over time, as indicated by the area under the disease progress curve ( $p < 0.05$ ). The results of this study indicate that temperature and rainfall are key factors in determining disease development, and ManKocide and Actigard foliar applications are highly effective in controlling the disease with 3 to 4 applications early in the spring season.

#### **Whole genome sequencing of *Xanthomonas perforans* identifies effectors that influence breeding strategies**

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The management of bacterial spot caused by *X. perforans* on tomato is a challenge due to historical changes in the pathogen populations. The availability of genomic sequence for hosts and pathogens provides new data for effector-based breeding strategies. We examined the stability and evolution of several effectors in *X. perforans*, based on genome sequence from representative strains collected in Florida since 1990. Effectors AvrXv3 and XopJ4 trigger resistance in tomato cultivars carrying the corresponding R genes. However, the Florida *X. perforans* population has shifted from tomato race 3 to tomato race 4, such that avrXv3 is non-functional in all tomato race 4 strains with mutations specific to different phylogenetic groups. The *X. perforans* effector genes *xopJ4* and *avrBs2* remain intact in all strains. The *avrBsT* gene is absent in race 3 strains collected in the 1990s, but present in race 3 strains collected in 2006 and the majority of race 4 strains. The conserved presence of XopJ4 and increased presence of AvrBsT among race 4 strains of *X. perforans* make them ideal targets for resistance breeding. Specific R-genes against these two effectors could be pyramided with Bs2 resistance to develop tomato cultivars with enhanced resistance against *X. perforans* for commercial production in Florida.

#### **Cybridization: A promising plant resistance for citrus canker control**

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Cybridization is a somatic hybridization system that enables the construction of novel nuclear and cytoplasmic genome combinations. In this work, cybridization was used to study the role of mitochondrial (mt) and chloroplast (cp) genes in resistance to *Xanthomonas citri* subsp. *citri* (Xcc), the cause of citrus canker. Putative cybrids were created through the fusion between nucellar callus cells of Meiwa kumquat (*Fortunella crassifolia*), a citrus canker resistant, and mesophyll cells of citrus canker susceptible grapefruit (*Citrus paradisi*). Six EST-SSR markers demonstrated that all clones have a grapefruit nuclear genome; however, four CAPS markers showed variation in the cytoplasmic genomes. In 123 cybrids, kumquat mt and cp were identified, and in 19 cybrids, kumquat mt and grapefruit cp were present. Eighty-two cybrid clones were evaluated by detached leaf assay (DLA), and clones that performed better in the DLA were evaluated by syringe inoculation of attached leaves. Q-PCR was carried out to quantify the Xcc population.

Cybrid clones showed a range of citrus canker resistance, but all clones produced a significantly lower number of lesions and a one log unit reduction of the Xcc population compared to grapefruit. Cybrid clones with grapefruit cp were more susceptible to Xcc compared to clones with kumquat cp. In addition to citrus canker resistance, these cybrids provide a valuable model to study the role of cytoplasmic genomes in plant disease resistance.

#### **Biological control of plant pathogenic bacteria**

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Bacterial diseases take a high economical toll in all areas of agriculture. Currently growers attempt to treat these outbreaks with copper compounds that have low efficacy and are detrimental to the environment. Nobactra brings a new concept to control a wide range of bacterial diseases, comprised of two key elements that when combined have a strong synergistic relationship. The first component is a cocktail of 10 antagonistic bacteria and the second component is a powder formulation of essential oils. The advantages of using a cocktail of bacteria rather than a single microbe include: a) consistency in a range of soil and climatic conditions; b) significantly lower chances of resistance development due to the multiple mechanisms of activity; c) wide spectrum of activity. The innovative oil formulation overcomes the high phytotoxicity of the essential oil and allows for effective use against bacterial diseases without harming the plant. Nobactra's solutions have shown high efficacy rates in the control of several diseases. The following are key examples: Potato scab (*Streptomyces* spp.) – seed treatment and soil treatment. *Tomato canker* (*Clavibacter michiganensis* subsp. *Michiganensis* - Cmm) Nobactra controls the distribution of Cmm in the greenhouse. Seed borne bacterial diseases (*Pseudomonas*, *Xanthomonas*, *Acidovorax*, *Erwinia*, Cmm.). Nobactra's products are officially registered in Israel.

#### **Investigation of the anti-bacterial activity of SERENADE® ASO against bacterial diseases of crops**

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Bacterial diseases of agricultural crops are costly with losses estimated at \$10 billion per year. Current management strategies often use a multifaceted approach, including biological control. The biological-based product, SERENADE® ASO, is a known tool in integrated foliar spray programs to combat fungal diseases in fruits and vegetables, based on fungal cell membrane disrupting properties of lipopeptide chemistry produced by *Bacillus subtilis* QST-713. In addition, SERENADE® ASO has proved helpful to growers in managing bacterial diseases, such as *Pseudomonas syringae* pv. *tomato* and *Xanthomonas campestris* pv. *vesicatoria*, causal agent of bacterial speck and spot, on crops such as tomato and pepper. Recent research using both *in vitro* and *in vivo* tools point to two main actions contributing to the antibacterial activity of SERENADE® ASO: a direct antibacterial effect from biological chemistry produced by QST-713 during fermentation and an indirect effect on the plant. Different approaches were applied to eliminate potential activity from living cells in SERENADE® ASO to examine the role of biological chemistry. Research with *Arabidopsis* treated with SERENADE® ASO showed up-regulation of several pathways associated with host defense, and microscopy studies using tomato seedlings revealed transient changes in the condition of stomata openings. Additional research is ongoing to better characterize the mechanism of SERENADE® ASO against plant pathogenic bacteria.

#### **Grass residues as a sustainable carbon source in application of anaerobic soil disinfestation for control of apple nursery replant disease**

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Studies were conducted to assess the efficacy of anaerobic soil disinfestation (ASD) for control of replant disease in an apple nursery setting. Treatments applied in a field trial conducted at an experimental orchard in Washington State included ASD using orchard grass residues (GR; 20 t ha<sup>-1</sup>) as the carbon source, Telone C35 (27 gal acre<sup>-1</sup>) and no treatment control (NTC). The apple rootstocks G.935, G.41, and M.9, with respectively decreasing level of tolerance to replant disease, were planted into each of six blocks per treatment with five replicates per rootstock. Post-treatment weed biomass analysis, rootstock growth analysis and rhizosphere fungal community analysis were conducted. Prior to tillage that preceded rootstock planting, ASD yielded significant weed suppression but weed biomass in fumigated plots was comparable to NTC. During the first growing season trunk diameter of G.41 and G.935 rootstocks were significantly increased by ASD compared to the NTC and it was comparable to fumigation treatment. ASD enhanced growth of M.9 relative to NTC but was lower than that for the fumigation. According to TRFLP analysis, different soil treatments possessed unique rhizosphere fungal communities. Based on this study, an increase of 5-25% gross revenue was projected using ASD based on market value of different rootstocks and sizes. ASD with grass is a potential viable soil-borne disease management practice for the apple nursery industry.

#### **Management of Fusarium wilt Tropical race 4 in bananas**

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*Fusarium oxysporum* f.sp. *cubense* Tropical race 4 (TR4) is one of the most important pathogens of banana which severely affects many banana varieties, including Cavendish. First identified in Taiwan, TR4 has spread rapidly over major banana production areas and has had a major impact on banana production. More recently it has been identified outside Southeast Asia in Mozambique, and in Jordan. The most recent incursion was detected in Australia's main banana growing region around Tully in North Queensland in March 2015. The continuous long distance dissemination of this fungus, as well as local spread to new plantations and fields, raises many questions concerning our ability for effective containment of this pathogen. The recent outbreak in Australia also highlighted a lack of understanding of many basic pathological and epidemiological facts of this important pathogen. Therefore, our aim was to improve detection and identification of the pathogen, destruction of infected plants, containment strategies and overall management of plantations. In the absence of high levels of resistance in economically acceptable banana varieties, containment may be a temporary but effective strategy to win valuable time to identify or develop banana varieties with high levels of resistance to Panama wilt TR4. A global program for evaluation of germplasm in multiple field sites linked with international collaborators is urgently needed.

#### **Pseudomonas sp associated with Smilax bona-nox display strong activity against Phytophthora spp**

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*Smilax bona-nox* is a perennial weed, which is found in natural landscapes and forests throughout the southwestern U.S. *S. bona-nox* displays disease tolerance and vigorous growth, which could be partially attributed to beneficial plant-associated microorganisms. In this report, we recovered 44 bacterial isolates from roots and shoots of *S. bona-nox*. Based on 16S rRNA gene sequencing, these isolates belonged to 14 different genera. Antimicrobial screens showed that two of these isolates, EA6 and EA14, displayed strong inhibition of *Phytophthora parasitica*. Phylogenetic analyses based on the *rpoD* gene revealed that EA6 and EA14 belonged to the genus *Pseudomonas*. EA6 and EA14 did not produce biofilm, but produced bio-surfactants, volatile hydrogen cyanide and sulfur compounds. Biochemical fractionation of the volatile and secreted products of these two isolates for identifying the bioactive compound(s) revealed that volatile and organic extracts did not display any anti-*Phytophthora* activity. In contrast, salt precipitation of the aqueous phase revealed strong anti-*Phytophthora* activity, which was abolished by autoclaving or proteinase K treatments. Protein fractionation analyses suggest that the antimicrobial activity of EA6 is likely due to synergistic interaction of a mixture of glucanolytic enzymes. In conclusion, these two *Pseudomonas* isolates can be used for organically controlling *Phytophthora* diseases in crops.

### **Efficacy of biopesticides on root diseases and pests in hydroponic production of vegetables**

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Soil-borne pathogens cause significant losses in hydroponic greenhouse vegetable production, even when fungicides and water treatments are employed. Several microbes and microbe-derived products protect plants from pathogens. Many new products are available but comparative efficacy data is lacking. This research evaluated biopesticide efficacy under commercial conditions to identify implications for IPM. A small scale hydroponic system was constructed to evaluate efficacy of 10 bio-pesticides on commercial greenhouse tomato and cucumber cultivars. The effect of cultivar, grafting, and substrate type on product efficacy was also determined. Products were applied as a drench at transplant to rockwool blocks and at transplant to coco or rockwool slabs. Products were compared to fungicide and water controls. Plants were challenged with *Pythium* spp. 3-5 days after product application and root rot, plant height and weight was evaluated after 3 weeks. Several products reduced root rot equal to or greater than the fungicide Previcur. Disease reduction varied depending on the substrate, time of year and rootstock genetics. The best performing products in the disease trials were tested for potential positive or negative effects on performance of greenhouse pests *Trialeurodes vaporariorum* and *Tetranychus urticae*. Results from this research will allow growers to make informed management decisions and derive maximum benefit from their investment in disease control products.

### **Organic rice disease management research in the United States**

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Organic rice production has steadily increased in the U. S. over the last 20 years, with a majority of acreage being grown in the southern region. Due to warm and long growing seasons in the region, diseases are among the most important factors threatening the sustainability and productivity of organic rice. Field studies were conducted for the first time in the U. S. to evaluate the efficacy of cultivar resistance, cover crop, fertility, tillage and biocontrol agent for management of rice diseases on organically managed land in Texas in 2009 to 2014. Among 20 cultivars evaluated, Tesanai 2 had the highest level of resistance to both narrow brown leaf spot (NBLs) and brown spot followed by XL723 and XL753. Production following the incorporation of clover winter cover crop resulted in a consistent reduction in NBLs and brown spot than following winter fallow or annual ryegrass cover crop. An application of the soil amendment Nature Safe or Rhizogen at 90, 150 or 210 kg N/ha significantly reduced NBLs and brown spot. Use of conventional tillage practice prevented the occurrence of straighthead on all 20 cultivars evaluated in the clover winter cover crop plots. A foliar application of Serenade Max significantly reduced NBLs and sheath blight, resulting in an increase in yield. Cultivar resistance, cover crop, fertility, tillage and biocontrol can be effective tools for management of diseases in organic rice production.

### **Elucidating the genetic basis of race differentiation in *Cercospora sojae* through genotype-by-sequencing approaches**

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*Cercospora sojae*, the causal agent of frog-eye leaf spot (FLS), is a widespread pathogen of soybean in the United States. Recently, a race structure was proposed for *C. sojae* based on susceptibility vs resistance responses of 12 soybean differential cultivars. We hypothesize that the delineation of races in *C. sojae* is attributable to variation in effector gene complements among individual isolates. To test this hypothesis, we devised a genotype-by-sequencing strategy for *C. sojae* to facilitate association mapping of loci associated with race structure. We developed a restriction-site associated DNA sequencing (RAD-seq) approach to cost-effectively genotype large numbers of *C. sojae* isolates, with the ultimate goal of identifying single nucleotide polymorphisms (SNPs) for association mapping. Bulk segregant analysis was performed with two pools of *C. sojae* isolates (48 isolates per pool, selected based on resistant or susceptible reaction on soybean cultivar UA5612). Ion Torrent PGM sequencing identified 15,158 SNPs, and candidate pathogenicity genes were identified based on differences in SNP density and non-synonymous SNPs between the resistant and susceptible pools. Elucidating the genetic basis of race structure in *C. sojae* will accelerate the development of DNA-based assays to differentiate races of the pathogen, and contribute to the fundamental understanding of genetic mechanisms underlying FLS.

### **RNA-Seq analysis of *stuA* mutants in *Fusarium verticillioides* indicates dramatic genomic wide transcriptional reprogramming**

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StuA, first discovered in *Aspergillus nidulans* and a member of the APSES class of transcription factors, regulates several essential developmental stages in fungi such as virulence, sporulation and toxin production in phytopathogenic fungi. *Fusarium verticillioides* (*Fv*), a maize phytopathogen, produces fumonisin mycotoxins in maize. Fumonisin-contaminated corn causes fatal toxicity in livestock, is associated with neural tube birth defects and growth stunting in children and is also a potential carcinogen to humans. Our objective is to explore the impact of the *stuA* homolog on morphogenesis and toxin production in *Fv*. *stuA* mutants were generated via *Agrobacterium*-mediated transformation of *Fv* with an OSCAR deletion construct. Characterization of the mutants indicate slower vegetative growth on solid media, reduced sporulation and macroconidiation. A cracked corn assay on maize kernels and virulence assay on maize seedlings revealed reduced fumonisin accumulation and reduced virulence respectively in *stuA* mutant infected maize as compared to the wild-type pathogen infection. To characterize the mutants transcriptomally, RNA-sequencing of three of the mutants and the wild-type *Fv* strain M3125 was done via Illumina NextSeq and the Tuxedo pipeline is being utilized for differential expression analysis. Preliminary RNA-seq analysis indicates major alteration of numerous transcription factors, transporters and signaling in the *stuA* mutants.

### **Enhancing our understanding of *Fusarium* maize stalk rot pathogenesis through gene association subnetwork module analyses**

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*Fusarium verticillioides* is an important stalk rot pathogen of maize. While a select number of genes associated with virulence have been characterized, our knowledge of the genetic network underpinning this mechanism is very limited. Previously, our lab identified a striatin-like protein, Fsr1, that plays a key role in stalk rot pathogenesis. To further characterize genetic networks downstream of Fsr1, we performed RNA-Seq with maize B73 stalks inoculated with *F. verticillioides* wild type and *fsr1* mutant. Datasets were first used to infer *F. verticillioides* co-expression networks. Subsequently, we used a computationally efficient branch-out technique, along with an adopted probabilistic pathway activity inference method, to identify functional subnetwork modules likely involved in *F. verticillioides* pathogenicity. Through our analyses, potential pathogenicity-associated subnetwork modules were identified, each consisting multiple interacting genes with coordinated expression patterns, but whose collective activation level is significantly different in the wild type and the mutant. In this study we selected three putative pathogenicity genes, *FvSYN1*, *FvEBP1* and *FvCYP1*, from three predicted subnetworks. We generated gene-deletion mutants and performed pathogenicity assays. Furthermore, qPCR assays were performed to determine whether these genes serve as a hub gene and impose critical regulatory control on associated genes in each subnetwork.

### **Genetic basis for pathogenicity in *Fusarium oxysporum* f.sp. *cepae* causing basal rot in onion**

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Basal rot of onion, caused by *Fusarium oxysporum* f.sp. cepae (FOC), is a soilborne fungus and part of the *F. oxysporum* species complex, which includes other important pathogenic *formae speciales* (f.spp.) adapted to particular crop hosts, as well as non-pathogenic isolates. The genetic basis for pathogenicity in *F. oxysporum* is poorly understood, but 'secreted in xylem' (SIX) genes have recently been associated with virulence in several f.spp. A set of 32 *F. oxysporum* isolates were tested for pathogenicity on onion and whole genome sequencing and PCR approaches used to detect putative effector genes. Seven SIX genes and two other putative effectors were identified in highly pathogenic isolates which were all absent in non-pathogenic isolates. Expression of SIX genes and other putative effectors increased *in planta* over 96 hours following inoculation of onion seedlings. Different SIX gene complements were also identified in other f. spp. but none were found in *F. avenaceum*, *F. proliferatum* or *F. redolens*, which have also been associated with basal rot. Preliminary results from a comparative analysis of genomes from pathogenic and non-pathogenic *F. oxysporum* isolates will also be described as well as the potential use of SIX genes as a diagnostic to enable quantification of FOC and assessment of disease risk in field or store.

#### **Quantitative pyrosequencing for rapid detection and quantification of *Aspergillus flavus* atoxigenic active ingredients in complex fungal communities**

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Use of atoxigenic *Aspergillus flavus* genotypes to displace aflatoxin producers is a highly effective method for reducing aflatoxin contamination in crops. However, there is a need for improved methods for rapid assessment of treatment efficacy and residual effects. To address this need, the current study designed twenty-four quantitative pyrosequencing assays for rapid post-harvest detection and quantification of *Aspergillus flavus* genotypes which serve as active ingredients in the biocontrol product Aflasafe™ that is registered for use in Nigeria. The fungi must be quantified in mixed fungal populations associated with crop surfaces. Sixteen of the assays are for individual genotypes and eight are for simultaneous detection and quantification of multiple active ingredients. Assay refinement with mixtures of DNA from target and non-target genotypes indicated both accuracy and sensitivity of the assays in detecting the active ingredients in fungal populations. Quantification of target alleles by single and multiple genotype assays was highly correlated with proportions of target DNA in the mixtures. Results from tests to validate the assays with DNA extracted from treated corn samples were not consistent among assays. The current results demonstrate that quantitative pyrosequencing is a robust and reliable tool for rapid detection, quantification, and monitoring of multiple genotypes within complex fungal communities associated with crops.

#### **Engineering mobile RNA in Carrizo to enhance plant defense responses to control citrus greening**

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Plants modulate plant defense responses against pathogens with both pro- and anti-programmed cell death (PCD) proteins. By suppressing expression of anti-PCD proteins, PCD can occur more rapidly and with stronger defense responses. There are many PCD triggers, including Reactive Oxygen Species (ROS) which activate plant defense signaling pathways over long distances. Citrus greening is caused by *Ca. Liberibacter asiaticus* (Las). Las produces a peroxidase that has been suggested to dampen the ROS signaling pathway. A gene silencing strategy was used to suppress an anti-PCD gene in Carrizo rootstocks. The expression levels of three citrus defense response genes (*NDRI*, *PR1* and *EDSI*) were significantly higher in silenced lines than in nontransgenic (NT) controls after inoculation with Las flagellin (flg22), used as a proxy for Las. Silenced Carrizo lines were also more tolerant to heat and cold stresses than NT controls. Challenge inoculations are currently underway using approach-grafted, Las infected citrus; all sampling was taken from leaves above the approach graft union. To date, three transgenic Carrizo lines failed to become infected over a 9 month challenge period as compared with two transgenic lines that became infected within two months. Following removal of the infected source tree, two NT scions that were consistently positive have now tested negative, possibly indicating movement of the silencing signal from transgenic rootstock to NT scion.

#### **Developing resistance to citrus Huanglongbing**

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Citrus Huanglongbing (HLB) has caused significant damage to citrus industries in Florida and Brazil. The disease is associated with a fastidious bacterium, *Candidatus Liberibacter asiaticus* (Las) and transmitted by psyllids. Development of HLB resistant rootstock and scion cultivars will be beneficial to the industry. Sources of HLB resistance are not known in the genus *Citrus*. We have demonstrated HLB resistance in *Eremocitrus* and *Microcitrus* which are closely related genera sexually compatible with *Citrus*. Naturally occurring hybrids of *Citrus* with *Eremocitrus* and *Microcitrus* parentage were exposed to the HLB pathogen under greenhouse conditions and determined to be HLB resistant in controlled experiments. Since the resistance from these non-*Citrus* genera appears to be heritable, we have generated about 800 mandarin and trifoliolate hybrids using *Eremocitrus* and *Microcitrus* as pollen parents. The hybrid seeds were raised in greenhouses in Florida and challenged by feeding with LAS-positive psyllids for four weeks. Preliminary results indicate the absence of Las in many hybrids. We are monitoring the putative resistant hybrid plants continuously by assaying for the presence of Las by qPCR and digital PCR, and also by observing the hybrids for expression of disease symptoms. We will confirm resistance after conducting no-choice psyllid feeding experiments.

#### **Proposed model for *Candidatus Liberibacter asiaticus* and solanacearum systemic invasion and multiplication in the psyllid host and vector**

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*Ca. Liberibacter asiaticus* and solanacearum are the causal agents of Huanglongbing and Zebra chip disease of citrus and solanaceous crops, respectively. Bacterial effectors that interact with psyllid vector proteins during invasion of and exit from the gut, and that facilitate entry into the salivary glands were identified using functional genomics, proteomics, yeast-2 hybrid (Y2H), co-immunoprecipitation (Co-IP), and electron microscopic analyses. *In silico* annotation and differential expression studies of psyllid nymphs and adults, and midgut and salivary glands identified transcripts and proteins with altered expression in response to *Ca. Liberibacter* infection. Differentially expressed transcripts were used as bait in Y2H studies to corroborate protein-protein interactions. Those positive by Y2H were subjected to verification by bait to prey co-transformation and Co-IP. Electron micrographs of the PoP midgut revealed the presence of CLso in the ventricular lumen, apical and basal epithelial cytosol, and in the filter chamber periventricular space. CLso were also prevalent in salivary gland pericellular spaces and head epidermal cell cytosol. Collectively, the results suggest a model for invasion in which *Ca. Liberibacter*- and prophage-encoded effectors manipulate the host endocytic pathway into a 'pathogen-mediated phagocytosis scenario' leading to bacterial exit from the gut into the hemocoel, and systemic invasion of other psyllid tissues and organs.

#### **Systemic infection of split root trees by *Candidatus Liberibacter asiaticus* suggests rapid movement between phloem sieve tubes**

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Huanglongbing (HLB; syn. citrus greening), caused by the phloem-limited bacterium *Candidatus Liberibacter asiaticus* (Las), poses one of the largest threats to citrus production. In ten years it has spread to most citrus trees in Florida. Citrus production in Florida is down >70% from the pre-HLB era. Las infects a tree when an infected *Diaphorina citri* (Asian Citrus Psyllid) feeds on phloem sap in a leaf. After infection of the leaf, Las rapidly colonizes the root system, but remains sectorial in the canopy. Canopy sectoring is thought to occur because of limited lateral connections of phloem sieve tubes.

To investigate if lateral movement of Las between sieve tubes is distance or tissue specific, root systems of grafted and seedling trees were split to three different heights: below crown, above crown, and above graft union (or equivalent on seedlings). The trees were then graft inoculated above one side of the split root system. Infection to both halves of the root system was monitored weekly. Contrary to our hypothesis; the graft union and crown do not play a unique role in lateral movement. Similar patterns of infection occurred in all tissue types of the grafted trees and seedling trees. Greater variability in the time between Las detection in the root systems on the inoculated side and the opposite side in all trees with the highest split suggests that vertical distance is important in lateral movement of the bacterium.

#### **Cultivar-specific wheat miRNAs during stripe rust infection in *Triticum aestivum***

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Wheat represents approximately 30% of the world's production of grain crops with more than 220 million cultivated hectares. Productivity at harvest is mainly governed by genetic composition and environmental factors influencing growth and development. Understanding the genetic factors that regulate these processes will help in developing varieties with better yield potential and disease resistance. MiRNAs are regulators of gene expression in eukaryotes that control myriad processes from development to abiotic and biotic stress responses. Using 12 small RNA libraries prepared from two wheat cultivars infected with stripe rust fungus (*Puccinia striiformis*), we identified 43 previously-known miRNAs, 50 novel variants of known miRNAs and 93 candidate novel miRNAs. Digital gene expression revealed 20 cultivar-specific miRNAs, 6 miRNAs differentially expressed between the two cultivars and 4 miRNAs responsive to stripe rust infection. These results were validated using RT-PCR and qRT-PCR. Using different target prediction algorithms, 69 wheat miRNAs were found to target fungal genes- a majority of which coded for small, secreted, cellular proteins. These results suggest a trans-kingdom regulation of gene expression potentially involved in the cultivar-specific resistance to stripe rust. Overall, this study contributes to the current repository of wheat miRNAs and provides novel information on the as yet uncharacterized roles for miRNAs in wheat host-pathogen interactions.

#### **Antifungal mechanisms of a plant defensin MtDef4 are not conserved between ascomycete fungi, *Neurospora crassa* and *Fusarium graminearum***

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Small cysteine-rich defensins are ubiquitous antifungal plant peptides which contribute to the innate immunity of plants. The plant defensin, MtDef4, inhibits the growth of the ascomycete fungi, *Neurospora crassa* and *Fusarium graminearum*, at micromolar concentrations. Previous studies have suggested that MtDef4 is transported into the cytoplasm of these fungi and exerts its antifungal activity on various intracellular targets. Here, we have investigated whether the antifungal mechanisms of MtDef4 are conserved in these fungi. We show that *N. crassa* and *F. graminearum* respond very differently to MtDef4 challenge. Membrane permeabilization is required for the antifungal activity of MtDef4 against *F. graminearum* but not against *N. crassa*. We find that MtDef4 is targeted to different subcellular compartments in each fungus. Internalization of MtDef4 in *N. crassa* is energy-dependent and involves endocytosis. By contrast, MtDef4 appears to translocate into *F. graminearum* autonomously using partially energy-dependent pathway. MtDef4 has been previously shown to bind to the phospholipid phosphatidic acid (PA). We provide evidence that the plasma membrane localized phospholipase D (PLD1), involved in the biosynthesis of PA, is needed for entry of this defensin in *N. crassa*, but not in *F. graminearum*. To our knowledge, this is the first example of a plant defensin which inhibits the growth of two ascomycete fungi via different mechanisms.

#### **Identifying genetic variation in the *Sorghum* PAMP response**

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*Sorghum* is an important grain, forage and biofuel crop with a fully sequenced genome and a variety of available genetic resources. In comparison to studies of Arabidopsis, very little is known about the innate immunity response of crop species, including sorghum. Such studies could contribute to efforts to create crops with enhanced disease resistance. The plant innate immunity system is triggered by plant recognition of conserved pathogen molecules, so called pathogen-associated molecular patterns (PAMPs). We measured PAMP induced production of reactive oxygen species (ROS) as a quantitative measure of the immunity response in more than 50 different *Sorghum* genotypes. In addition, we also analyzed the production of nitric oxide (NO) as another measure of the plant response to PAMP addition. The results showed a significant variation among the phenotypes with a few responding very strongly with others showing essentially no response. This variation provides the basis for additional experiments focused on defining specific genetic loci that control the *Sorghum* PAMP response. We are also conducted a transcriptomic analysis of the *Sorghum* PAMP response with the goal to define expression QTL (eQTL) related to pathogen resistance. The goal is to use the information obtained to aid breeding efforts to increase biotic stress resistance in *Sorghum*.

#### **Understanding quantitative disease resistance in rice: Defense response gene regulation and promoter patterns**

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The potential yield of rice worldwide is reduced by one third due to disease. The extensive work in breeding rice varieties that harbor resistance genes has helped to mitigate this damage. The method is effective, but the selective pressure of a single host resistance gene can be overcome after only a few generations. Enhancing the expression of basal defense response (DR) genes that are activated against a broad spectrum of pathogens could be a more sustainable approach to breeding for resilience. DR gene promoters can affect timing and intensity of expression, and rice varieties with good basal resistance have been shown to contain polymorphisms that are beneficial in the activation of the defense response. Recently, the genomes of a diverse panel of 3000 rice varieties were sequenced. This information, along with transcriptomic analyses of resistant interactions, were utilized in this study to uncover the genome-wide patterns in DR gene promoters. These patterns are potential cis-elements, and DR gene-specific combinations of these elements in multiple loci infers their functionality in fine-tuning regulation during a resistant host-pathogen interaction. These results could refine marker assisted breeding for a stable, broad spectrum resistance.

#### **COS-OGA stimulates plant innate immunity in a cumulative process that involves salicylic acid**

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COS-OGA is a new patented active substance for plant protection registered in Europe against powdery mildew. COS-OGA contains chitosan oligomers (COS), plant non self-molecules combined with pectin-derived oligogalacturonides (OGA), plant self-molecules. COS-OGA mimics a plant-fungus interaction: during plant infection, fungal pathogens deacetylate their cell wall chitin into chitosan to escape plant chitin receptors. Chitosan fragmentation by plant enzymes then yields polycationic COS molecules while plant cell wall degradation by fungal polygalacturonases releases polyanionic OGA molecules. The plant defense stimulation was investigated on tomato plants after several foliar sprayings of COS-OGA. A proteomic study on leaves revealed that the elicitor treatment led mainly to an overexpression of Pathogenesis-Related proteins, among which P69 subtilisin-like proteases. Gene expression study by qRT-PCR revealed that COS-OGA significantly upregulated transcripts of salicylic acid (SA)-responsive genes while transcripts linked to ethylene and jasmonic acid pathways were not affected. The activation of SA-dependent plant defense reactions was

confirmed by SA quantification in the leaves. The results showed an increase of free SA levels in leaves, proportional to the number of COS-OGA applications. Finally, repetitive COS-OGA sprayings efficiently protected greenhouse-grown tomato against *Leveillula taurica*, with 90% leaf protection in terms of severity.

#### **Degradation of the master regulator of plant defense NPR1 by the type III effector HopAB2**

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The plant hormone salicylic acid (SA) is required for systemic acquired resistance (SAR). NPR1, as an SA receptor, is required for SA-mediated plant defense. The expression of over 97% of SA-responsive genes is dependent on NPR1, therefore, NPR1 functions as a master regulator of plant defense. Many studies have shown that pathogen effectors suppress or disrupt MAMP-triggered immunity (MTI) and cell death. There are also isolated studies, which suggest that effectors suppress or disrupt SA-mediated plant defense. In order to provide strong and direct evidence on the targeting of SA-mediated plant defense by pathogen effectors, we tested the interactions between the SA receptor NPR1 and all type III effectors from the bacterial pathogen *Pseudomonas syringae* in yeast two-hybrid assays. Interestingly, we found that the type III effector HopAB2 interacts with NPR1 only in the presence of SA or its active analogs. Using Agrobacterium co-infiltration assays, we found that HopAB2 degrades GFP-tagged NPR1 protein, dependent on its E3 ligase activity and through the 26S proteasome. Using Dexamethasone (DEX)-inducible *Arabidopsis* transgenic plants expressing the hopAB2 gene, we found that HopAB2 degrades NPR1 native protein. Similar to *npr1-2* mutant plants, these transgenic *Arabidopsis* plants show sensitivity to SA-induced toxicity and are comprised in SAR. Our studies provide solid evidence that HopAB2 disrupts SA-mediated plant defense by degrading NPR1.

#### **Resistance of Carolina Gold Select rice to African strains of *Xanthomonas oryzae* pv. *oryzicola* is triggered by inactivated TAL effectors**

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The rice pathogen *Xanthomonas oryzae* pv. *oryzicola* causes bacterial leaf streak of rice, a disease for which no single-gene resistance is available. The bacterium produces numerous transcription activator-like (TAL) effectors that activate host susceptibility genes. There are two common rice resistance mechanisms against TAL effectors: DNA polymorphisms that allow escape of TAL effector binding, and placement of TAL effector binding sites to cause specific TAL effectors to activate resistance genes. We found that the heirloom rice variety Carolina Gold Select has a third form of resistance, triggered by diverse TAL effectors. The resistance was triggered in a manner independent of the transcriptional activation domain and of the composition of repeat variable diresidues that specify host DNA target sequences. While the TAL effector central repeat region was critical to resistance, an effector with only 3.5 central repeats was sufficient for resistance activation. The resistance was mapped to a single dominant locus, *Xo1*, on chromosome 4. The *Xo1* interval confers complete resistance to strains in the African clade of *X. oryzae* pv. *oryzicola*, representing the first dominant resistance locus against bacterial leaf streak in rice. The TAL effector triggered resistance phenotype has strong similarity to that conferred by the tomato resistance gene *Bs4*, suggesting that monocots and dicots have converged on the structure of TAL effectors as a pattern triggering resistance.

#### ***Xanthomonas* effector kinase XopAU promotes disease symptoms by activation of host MAPKK**

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*Xanthomonas euvesicatoria* (*Xcv*) is the causal agent of bacterial spot disease of pepper. *Xcv* pathogenicity depends on a type III secretion system that delivers effector proteins into host cells to manipulate host signaling and promote disease. Transient expression of the *Xcv* effector XopAU in host and non-host plants promoted typical defense responses including phosphorylation of MAPKs, accumulation of pathogenesis-related (PR) proteins and elicitation of cell death. Genetic analysis of XopAU by insertional mutagenesis and overexpression revealed a role for this effector in the development of chlorotic disease symptoms. XopAU encode a catalytically active protein kinase and this activity is required for its biological function. Protein-protein interaction studies demonstrated that XopAU physically interacts with the plant immunity-associated MAPKK MKK2. In addition, MKK2 was shown to be directly phosphorylated by XopAU *in vitro* while expression of XopAU *in planta* promotes an increase in the phosphorylation of MKK2. Silencing of MKK2 or overexpression of catalytically inactive MKK2<sub>K99R</sub> in *Nicotiana benthamiana* reduced XopAU-mediated cell death and MAPK phosphorylation while silencing of MKK2 in pepper attenuated the *Xcv*-mediated chlorotic disease symptoms. Together, this study indicates that XopAU contributes to *Xcv* disease symptoms by manipulating host MAPK signaling through activation of MKK2.

#### **Dissecting the function and downstream targets of *CsLOB1* in citrus**

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Citrus canker caused by *Xanthomonas citri* pv. *citri* (*Xcc*) is a devastating disease affecting most citrus species. PthA4, one of *Xcc* Type III secretion system effectors, is responsible for citrus canker pustule formation on citrus leave. PthA4 binds to the effector binding element in the promoter of *CsLOB1* to activate its gene expression, which subsequently activates genes responsible for pustule formation. *CsLOB1* has not been functionally characterized and it remains unknown how *CsLOB1* controls its downstream genes. *CsLOB1* was highly expressed in the citrus stem phloem and cambial zone in wild-type plants. We aim to study the function of *CsLOB1* and identify the putative downstream target genes of *CsLOB1*. To observe the physiological effect by suppressing *CsLOB1* expression, a Citrus Tristeza Virus-based RNAi vector, engineered with truncated anti-sense *CsLOB1*, was constructed for Virus-induced Gene Silencing (VIGS). Suppression of *CsLOB1* via VIGS caused decreased stem diameter and retarded plant growth. To further confirm this result, Agrobacterium-mediated transient gene expression method is being used to reveal the *CsLOB1* function during pustule formation post *Xcc* infection on citrus leave using small hairpin (shRNA) and artificial microRNA methods. Meanwhile, Dexamethasone-inducible *CsLOB1* over-expression transgenic plants were constructed to identify the putative downstream genes of *CsLOB1*.

#### **Trapping conidia spores of *Podosphaera aphanis* in high tunnels and field strawberry plots**

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Strawberry Powdery Mildew (SPM) caused by *Podosphaera aphanis* can be severe on susceptible cultivars, especially in tunnel and glasshouse production. Epidemics of SPM are largely driven by intense sporulation and conidia dispersal, which are two highly relevant factors for management strategies. The objective of this study was to evaluate SPM aerial conidia spore concentrations in plastic tunnel and field environments of Florida. A 7-day recording volumetric spore trap was installed in both the field and high tunnel plots during the 2015-16 growing season. Airborne conidial dose at each site was recorded hourly for 5 weeks, as was temperature, relative humidity, leaf wetness and rainfall. In both plots, spore counts followed a diurnal pattern, with a peak in aerial concentrations between 10 am and 2 pm. Cumulative airborne dose in the tunnel (1488 spores/m<sup>3</sup>) was notably higher than in the field (82 spores/m<sup>3</sup>). Several coincident factors likely contribute to the airborne dose in tunnel growing systems, including an inherently more favorable environment for sporulation and infection, and subsequent increased disease severity. However, factors such as residence time of conidia

within the system after becoming airborne, and effects of visible light and UV radiation on inoculum production and dispersal may also contribute to higher inoculum dose. Further work is being conducted to explore the effects of various environmental factors on conidia production and dispersal.

#### **Detection and quantification of *Bremia lactucae* by spore trapping and quantitative PCR**

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*Bremia lactucae* adversely affects marketability of lettuce. The disease has been managed with a combination of host resistance and fungicide applications with mixed success over the years. Fungicide applications are routinely applied under the assumption that inoculum is always present during favorable environmental condition. This approach could lead to fungicide resistance in *B. lactucae* populations. Detection and quantification of airborne *B. lactucae* near lettuce crops may lead to a more judicious timing of fungicide applications. For specific detection of *B. lactucae*, we designed a qPCR assay based on a target sequence in *B. lactucae* mitochondrial DNA. PCR tests using DNA from 83 geographically diverse isolates, representing 14 *Bremia* spp., and amplicon sequencing confirmed that the primers developed for the TaqMan assays are species-specific and only amplify template from *B. lactucae*.  $C_q$  values  $>35$  were considered as nonspecific but a single sporangium could be detected at a  $C_q$  of 34. The correlation ( $R^2$ ) for regression between sporangial density derived from flow cytometry and  $C_q$  values derived from the qPCR was 0.86. The assay was deployed using spore traps in the Salinas Valley, where nearly half the US lettuce is produced. The sensitivity of the *B. lactucae*-specific assay enabled detection of the pathogen within the two-week lettuce free period, and indicated that the assay can be useful for predicting inoculum load for timing of fungicide applications.

#### **Timing of primary inoculum release by *Monilinia vaccinii-corymbosi* infecting blueberries in the US Pacific Northwest**

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Mummy berry, caused by *Monilinia vaccinii-corymbosi*, infects highbush blueberry (*Vaccinium corymbosum*) and causes severe economic losses in the Pacific Northwest (PNW). Control is mainly based on calendar-based fungicide sprays. The primary inoculum source is ascospores produced from apothecia that develop on mummified berries, or pseudosclerotia, that overwinter on the soil surface. Timing of apothecial development and ascospore release varies among locations and seasons, and differences in environmental factors may be why control programs are not always effective. This study aimed to identify environmental factors involved in the timing of primary inoculum release in the PNW, and develop a predictive model for ascospore release. Apothecial development was monitored in a severely affected field from 2008-2013 in Corvallis, Oregon and in two fields in northwestern Washington during the 2015 season. Data for eleven environmental factors was obtained from in-field weather stations and Washington State University's AgWeatherNet. Soil temperature, solar radiation and precipitation were the most significant factors influencing apothecial development demonstrated by a generalized linear model regression analysis. A forecasting model developed to predict primary inoculum release is being validated for PNW locations during the 2016 season. Outcomes of this research will improve the timing of disease management practices and overall control of mummy berry in the PNW.

#### **Plant pathogens were collected over Florida up to 3500 m by a wing-mounted dust collection system called DART (Dust Atmospheric Recovery Technology)**

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Asian dust plumes deliver up to 64 million tonnes of dust over the NW of the USA, and African dust storms deliver over 50 million tonnes of dust over Florida each year. Recent studies have demonstrated that plant pathogens associated with Asian and African aerosols can be transported to the USA in naturally occurring dust storms. In order to initiate a long-term monitoring program of plant pathogens in Asian and African dust plumes, we have developed a dust collection system called DART (Dust Atmospheric Recovery Technology). The DART dust sampler was mounted on a F104 Starfighter jet and separately on a T6 Texan propeller driven airplane and test flown over FL in 2013-2014. The DART system utilizes a high-volume pump to pass air through 4 separate filtration units where both aerosols and microbial cells are captured. Flow rates through filters are directly correlated to increased air speed, and are inversely correlated to increased altitude. The DART dust sampler has performed nominally up to 7600 m, 0.92 Mach, and 3.5 +G's. During initial test flights in 2013 and 2014, fungi recovered from the lower troposphere over FL (up to 3500 m) contained plant pathogens including species in the genera: *Acremonium*, *Aspergillus*, *Cladosporium*, *Curvularia*, and *Fusarium*. Numbers of recovered fungi, but not bacteria, increased significantly when 5 or 10  $\mu$ m filters were used in the DART system compared to filter pore sizes  $\leq 2 \mu$ m.

#### **Validation of the CDM ipmPIPE Forecasting System: Relating Aerial Transport of Pathogen Spores to Outbreaks of Cucurbit Downy Mildew**

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Cucurbit downy mildew (CDM) caused by *Pseudoperonospora cubensis* is considered the most damaging disease of cucurbitaceous crops worldwide. Sporangia produced by the pathogen are carried by wind currents and deposited on host plants following rain events. The CDM ipmPIPE forecasting system integrates data on current disease outbreaks and trajectories of sporangia transport from source fields to forecast the risk of new disease outbreaks in the eastern United States. The forecasting system was validated by relating the predicted risks of disease outbreak to the presence or absence of sporangia after a rain event and the onset of disease symptoms in the field. Rainwater samples from eight states, AL, GA, LA, NC, NY, OH, PA, and SC were collected from planting to the first report of disease during the 2013 to 2015 seasons. The presence of sporangia was then analyzed using primers specific to the pathogen. The pathogen was detected in 4-30% of the 203 samples collected from 2013 to 2015. Presence of sporangia in rainwater samples correctly classified disease onset in 67%, 81% and 78% of the cases, while the forecasting system correctly predicted disease onset in 75%, 85% and 60% of the cases in 2013, 2014 and 2015, respectively. Predictions from the forecasting system and presence of sporangia in rainwater samples were in agreement in 60-87% of the cases analyzed except in 2015 when presence of sporangia in rainwater samples were detected infrequently.

#### **Rapid LAMP diagnostics for detection and disease alerts of *Phytophthora infestans***

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*Phytophthora infestans*, causal agent of the Irish potato famine, is a threat to food security globally and an important pathogen in the US on tomato and potato. In 2009, late blight epidemics in the eastern US were the worst in modern history due to widespread inoculum distribution from infected tomato transplants and favorable weather. That year the US-22 lineage was identified as the dominant lineage. USABlight ([www.usablight.org](http://www.usablight.org)) was launched in 2011 to improve disease detection and communication between researchers, growers, the industry, and the public about late blight. A total of 1094 late blight reports were recorded between 2011-2015. Of these, 64% were from tomato and a third primarily in the NE US. A shift in the dominant clonal lineage from mefenoxam sensitive US-22 to US-23 occurred and 82% of genotyped strains were US-23. Disease outbreaks begin each year in Florida winter tomatoes. Spread through movement of tomato fruit is suspected. We developed a loop-mediated isothermal amplification (LAMP) assay specific for detection of *P. infestans*. The assay can be run in the field at 65C, needs no thermocycling, and can detect 100-10 pg/ul of pathogen DNA. A prototype mobile reader developed by Mobile Assay has been used for in-field detection using a SYBR green stain and hand-held tablet. This mobile reader and LAMP assay is being tested in both the US and East Africa to improve pathogen detection and will be used to develop an Afrblight tracking database.

#### **LAMP lights the way: Early detection of airborne inoculum of *Magnaporthe oryzae* in turfgrass fields**

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A host-specialized form of *Magnaporthe oryzae* (*Lolium* pathotype, *MoL*) is the causal agent of Grey Leaf Spot (GLS), a detrimental disease of perennial ryegrass. Early diagnosis of this disease is crucial for making timely management decisions. A pathogen-specific quantitative loop-mediated isothermal amplification (LAMP) assay, that is able to detect as low as 25 spores of the pathogen, was developed in our laboratory to monitor GLS airborne inoculum. To assess the suitability of the method for field use, two perennial ryegrass plots were artificially inoculated with pathogen-infested dried grass clippings at The Ohio State University Turfgrass Research Center in the summer of 2015, and five continuously running custom impaction spore traps were placed in each plot. Sampling units were replaced daily and tested with the developed qLAMP assay while plots were monitored for symptom development. Results confirmed that the qLAMP assay-trap system was able to detect the pathogen's inoculum up to two weeks before symptoms developed in the field. The implementation of this assay by practitioners has the potential to be a useful decision support tool to guide initiation and timing of fungicide applications for GLS management.

#### **Detection of the select agent *Rathayibacter toxicus* using recombinase polymerase amplification coupled with a lateral flow device**

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*Rathayibacter toxicus* is a gram-positive plant pathogen responsible for a lethal disease of livestock in Australia. An easy, sensitive in-field detection method would enhance capabilities for port of entry inspections and biosecurity surveillance surveys of annual ryegrass (*Lolium rigidum*) hay and seed. Recombinase polymerase amplification (RPA) is a fast and sensitive isothermal DNA amplification and detection technology. Long forward and reverse primers (32 bp) and a long probe (49 bp) labelled with FAM were designed using gene involved in toxin production. TwistDX kit was used to amplify the target DNA (at ~37°C) and a lateral flow device used to visualize the amplified products. RPA primers and probes targeting the ITS region of the host genomes were designed for use as internal controls, to enhance the reliability and accuracy of the assays. RPA assays were tested against inclusivity and exclusivity panels for high specificity and accuracy. In-field validation in South Australia confirmed the efficiency and sensitivity of the RPA assays; detected the *R. toxicus* from infected samples. The proposed RPA method will enhance diagnostics, surveillance, disease management, and in-field biosecurity decisions.

#### **Development of a rapid AmplifyRP Acceler8 test for grapevine red blotch-associated virus in grapevines**

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Grapevine red blotch-associated virus (GRBaV) is of unprecedented concern to the grape industry since its discovery in 2011. A rapid, reliable, sensitive, and user-friendly test is needed to quickly identify GRBaV-infected grapevines and facilitate their timely removal from vineyards. An AmplifyRP Acceler8 detection kit was developed for GRBaV for use both in laboratories and vineyards. The test is performed in a disposable detection chamber for 20 minutes using crude grapevine extracts. The test specifically detects up to 11 copies of GRBaV genomic DNA in a matrix of healthy crude extract. The test has no cross reactivity to host plant tissues and grapevine-infecting pathogens including arabis mosaic virus (ArMV), grapevine fanleaf virus (GFLV), grapevine leafroll-associated virus 1 (GLRaV-1), grapevine leafroll-associated virus 2 (GLRaV-2), grapevine leafroll-associated virus 3 (GLRaV-3), grapevine leafroll-associated virus 4 strain 5 (GLRaV-4 strain 5), grapevine fleck virus (GFkV), tomato ringspot virus (ToRSV), tobacco ringspot virus (TRSV), *Xylella fastidiosa*, and *Botrytis cinerea*. The pellets contain all the necessary reaction components and are stable for a long period of storage even at 37°C. The test has been validated using both viral DNA and crude plant extracts as templates.

#### **An Integrated Microfluidic Device for Rapid Plum Pox Virus Enrichment and Detection**

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Rapid and sensitive detection is highly desirable for plant viral pathogens. However, plant samples with low virus concentrations pose significant challenges for early detection. We present a miniature disposable device, carbon nanotube size tunable enrichment microdevice (CNT-STEM), that is capable of rapidly concentrating and purifying plum pox virus (PPV) from leaf tissues. This microdevice enriches PPV in the sample by employing physical size-based exclusion. The device consists of patterned carbon nanotube (CNT) arrays and a microfluidics channel made of polydimethylsiloxane (PDMS). The microdevice captures PPV from homogenized leaf samples inside the CNT nanostructure while most of the host contaminants are removed. After elution, the PPV enriched sample is subjected to real-time RT-PCR. The procedure takes less than 2 hours, significantly faster than standard immunocapture virus enrichment procedure. The results showed that the microdevice increased analytical sensitivity of PPV real-time RT-PCR by up to 100 fold compared with the current USDA standard immunocapture sample preparation method. The microdevice could enable early detection of PPV by enriching virus particles directly from plant samples. The technology can also serve as a platform for enrichment of other plant viral pathogens.

#### **Aspergillus communities contaminating red chilies with aflatoxins**

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Aflatoxins are carcinogenic, immunosuppressive metabolites that cause health concerns across the globe. Several *Aspergilli* have been implicated in aflatoxin contamination events with some aflatoxin-producers associated with specific crops. Red chilies (*Capsicum spp.*) are produced in warm regions that favor aflatoxin producers. Aflatoxin in red chilies may result in market loss and even crop destruction. In order to gain insight into causal agents of aflatoxin contamination of red chilies, naturally contaminated chilies from markets in Nigeria and the US were examined. The *A. flavus* L morphotype



was the most frequently isolated member of *Aspergillus* section *Flavi* (89%). Fungi with S strain morphology were also detected in chilies from both countries (10%). Multi-gene phylogenetic analyses of partial gene sequences for nitrate reductase (*niaD*, 2.1 kb) and aflatoxin pathway transcription factor (*aflR*, 1.8 kb) resolved S morphotype fungi into three distinct clades. Fungi that produced only B aflatoxins formed a single clade with reference *A. flavus* regardless of L or S morphology. All S morphotype fungi from chilies purchased in Nigeria produced both B and G aflatoxins and were assigned to either *A. minisclerotigenes* or the previously described unnamed taxon S<sub>BG</sub> on the basis of phylogenetic affinities; over 60% of these fungi were assigned to *A. minisclerotigenes*. The results suggest the etiology of aflatoxin contamination of chili is complex and may vary with region.

#### **Diversity of the *Aspergillus* section *Flavi* S morphotype in Mozambique**

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Aflatoxins are potent mycotoxins that contaminate foods and feeds worldwide. These cancer causing toxins also impair development and suppress immune systems. In Mozambique rejection of groundnuts from export markets and high prevalence of liver cancer are linked to aflatoxins. To improve understanding of contamination in Mozambique, aflatoxin producers were compared with known causal agents from other regions. Since, *Aspergillus* section *Flavi* isolates with S morphology are linked to severe contamination in North America and East Africa, relationships of S morphology aflatoxin producers to previously described fungi were characterized. Partial sequences of aflatoxin transcription factor (*aflR*, 2.1 kb) and nitrate reductase (*niaD*, 2.1 kb) genes were compared among S isolates from Mozambique, Kenya, Benin, and USA. Phylogenetic analyses sorted 200 S isolates from Mozambique into four clades. Isolates in the first clade produced only B aflatoxins and were closely related to fungi previously associated with lethal aflatoxicosis in Kenya. The other three clades produce both B&G aflatoxins. One of these contained *A. minisclerotigenes* and another was sister to the Kenya isolates. The third B&G clade is divergent from but most closely related to both the NRRL isolate A-11612 and fungi from West Africa belonging to an unnamed taxon referred to as S<sub>BG</sub>. Fungi in all four clades produce high aflatoxin concentrations and are potentially important causes of crop contamination.

#### **RNAseq analysis reveals oxidative stress responses of *Aspergillus flavus* are related to stress tolerance and aflatoxin production**

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Aflatoxin contamination by *Aspergillus flavus* is exacerbated by drought stress in the field. Given that reactive oxygen species (ROS) both accumulate in plant tissues during drought and can stimulate aflatoxin production *in vitro*, we examined the responses of toxigenic isolates of *A. flavus* to oxidative stress focusing on secondary metabolite production using whole transcriptome sequencing. We examined two high toxin producing isolates, Tox4 and AF13, and one moderate producer, NRRL3357, in aflatoxin conducive yeast extract sucrose medium amended with various levels of H<sub>2</sub>O<sub>2</sub>. The high producers exhibited fewer differentially expressed genes (DEGs) than the moderate producer in response to increasing stress. Altered aminobenzoate degradation expression along with altered conidiation and growth also suggests reduced developmental rates in the isolates caused by oxidative stress. Aflatoxin genes were upregulated in response to increasing stress along with genes encoding aflatrein and kojic acid biosynthesis. Additional changes in primary metabolic genes also indicates adaptations to increasing stress. Given that the number and diversity of DEGs observed correlated with previously observed aflatoxin production and oxidative stress tolerance for each isolate, these data suggest that secondary metabolite production is involved in *A. flavus* oxidative responses. Continuing proteomic analyses using gel and iTRAQ methods are underway to confirm the transcriptome results.

#### **Hypoxia tolerance as a factor in *Aspergillus flavus* invasion and toxin contamination of developing maize seeds**

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*Aspergillus flavus* predominantly colonizes the embryo and peripheral tissues (e.g., pericarp and the aleurone layer) of maize seeds but shows limited or late invasion of the endosperm. This pattern correlates with the O<sub>2</sub> levels in seed tissues and is suggestive of a role for hypoxia tolerance in the successful invasion of the fungus in seeds. This proposal was tested by comparing *A. flavus* isolate responses to O<sub>2</sub> deprivation *in vitro* with those in field inoculated seeds. Hypoxia (3 to 5% O<sub>2</sub>) allowed conidial germination but retarded radial growth and inhibited conidial and sclerotial development. Anoxia imposed suspended animation in most isolates. Growth differences among isolates correlated with biomass differences in infected kernels. Gene expression studies showed that canonical hypoxia genes (e.g., *pdc1*) were induced, while key development genes (e.g., *brlA*) were strongly suppressed by O<sub>2</sub> deprivation. Aflatoxin (AF) levels were relatively high in inoculated kernels, although O<sub>2</sub> deprivation *in vitro* decreased AF content in most isolates. Oleic acid addition to the growth medium enhanced toxin levels under hypoxia, consistent with high AF levels observed in seed. Isolate differences in growth, sporulation, gene expression and AF accumulation patterns correlated with their responses during seed colonization, implicating hypoxia tolerance as a key virulence factor in *A. flavus*.

#### **Relationship between invasive brown marmorated stink bug and fumonisin contamination of field corn in the Mid-Atlantic**

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Invasive brown marmorated stink bug (BMSB) feeding damages a variety of crops including field corn. In 2013, a survey of BMSB-infested corn fields in VA indicated BMSB damage is correlated with fumonisin contamination. The objective of the current study was to quantify the relationship between BMSB injury and fumonisin contamination in field corn. Field trials were conducted in DE, MD, and VA in 2015. Ears were covered with mesh bags at tasseling to prevent insect damage. BMSB treatments were 0 or 4 adults applied to bagged ears, and *Fusarium* treatments were water or a spore suspension of *F. verticillioides* applied to ears at the R3 growth stage. Treatments were arranged in a randomized complete block factorial design. At maturity, ears were harvested and stink bug damage, *Fusarium* infection, and fumonisin concentrations were assessed. Stink bug damage and *Fusarium* infection were significantly different among locations, but there was not a location by treatment interaction. Treatments with BMSB had more damaged kernels compared to those without BMSB. Both BMSB and *Fusarium* treatments increased *Fusarium* infection of kernels. However, only the BMSB treatment influenced fumonisin concentrations. Results suggest BMSB increases *Fusarium* infection and fumonisin contamination in field corn. Further studies are needed to understand mechanisms by which BMSB increases fumonisins, and to develop management strategies to mitigate impacts of BMSB on field corn in the region.

#### **Exploration of biofilm formation and its potential link to virulence in the Goss's wilt pathogen *Clavibacter michiganensis* subspecies *nebraskensis***

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Goss's wilt is a bacterial disease of corn that has re-emerged in the Corn Belt of the United States since 2006 and can cause up to 50% yield loss in extreme cases. Management of the disease is difficult, and little is known about the interaction of the causal bacterium *Clavibacter michiganensis* subsp. *nebraskensis* (*Cmn*) with its host. Bacterial cells are known to form simple to complex aggregations, termed biofilms, on biotic and abiotic surfaces, and biofilm formation contributes to the virulence of several plant pathogenic bacteria. We examined the occurrence of biofilm formation in *Cmn*, and the potential linkage of this phenotype to virulence. A total of 19 *Cmn* strains were screened for biofilm formation using two in vitro methods, quantification of biofilm formation on glass coverslips and scanning electron microscopy evaluation of biofilms produced on gold mesh grids. These strains were screened for virulence using stab and cut inoculation techniques on Goss's wilt-susceptible corn hybrids and rated for disease symptoms 10 dpi. Results from these assays indicated variation in biofilm formation ability among strains. There did not appear to be a correlation between biofilm formation and virulence of the pathogen, suggesting that biofilm formation may play other roles in *Cmn* ecology.

#### **The negative regulatory function of *tepR* for the virulence of *Burkholderia glumae* in rice is exerted via the quorum-sensing master regulator gene *qsmR***

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*Burkholderia glumae* causes bacterial panicle blight of rice, which is a globally emerging rice disease. The quorum-sensing system mediated by *tofl* and *tojR* plays a key role in the regulation of major virulence factors, such as toxoflavin (a phytotoxin), lipase and flagella. *tepR*, a newly found regulatory gene of *B. glumae*, negatively regulates multiple virulence factors including toxoflavin, lipase and exoprotease. In this study, a comparative RNA-seq analysis and a series of genetic research were performed to characterize the global regulatory function of *tepR* in *B. glumae*. The RNA-seq analysis revealed that none of the known regulatory genes required for toxoflavin production (*tofl*, *tofM*, *tofR*, *toxJ*, and *toxR*) was differentially expressed between the wild type (336gr-1) and its *AtepR* derivative (LSUPB401), while the structural genes for the toxoflavin biosynthesis and transport were more highly expressed in the *AtepR* background. Similar results were also obtained from quantitative reverse transcription PCR experiments, which validated the RNA-seq analysis. Likewise, expression of *tepR* was not affected by the *tofl/tojR*-mediated quorum sensing system. Interestingly, *qsmR*, a key regulatory factor for flagellum biogenesis, was found to be upregulated in the *AtepR* strain and essential for both toxoflavin production and exoprotease activity. These results suggest that *tepR* exerts its regulatory functions on toxoflavin and exoprotease through *qsmR* in this pathogen.

#### **Research on Bacterial Endophytes at the Institute for Advanced Learning and Research**

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Bacterial endophytes live inside plants and represent a largely unexplored resource for enhancing sustainable agricultural production. They are also recognized as an untapped reservoir of novel natural products. We have demonstrated significant growth promotion of the bioenergy crop switchgrass by bacterial endophytes (*Burkholderia phytofirmans* PsJN and *Pantoea agglomerans* PaKM) under *in vitro*, growth chamber and greenhouse, as well as field conditions, particularly in low fertility soil. Currently, we have established a library of 225 bacterial endophytes isolated from various environments and characterized their abilities in N fixation, P solubilization and auxin production, as well as ACC deaminase activities. Many endophytes exhibited one or several of these plant beneficial traits. In the agricultural sector, researchers are exploring growth enhancement and disease resistance of crops important to the region such as tobacco, grapevine and tall fescue and testing both individual microbes and consortia for possible applications. In the environmental sector, scientists are using switchgrass and bacterial endophytes able to breakdown toxic organic compounds to remediate polychlorinated biphenyls (PCBs) in a contaminated waste water overflow pond and also to reduce PCBs in storm water. In the natural product sector, researchers are searching for unique natural products with wide-ranging applications as antibiotics, antivirals and anticancer agents.

#### **Genome analysis of *Rathayibacter toxicus* strain WAC3373 from Western Australia: Sequencing, assembly and annotation**

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Annual ryegrass toxicity (ARGT) is an often fatal disease of livestock caused by *Rathayibacter toxicus*, a toxin producing, nematode-vectored (*Anguina funesta*) gram positive bacterium. *R. toxicus* is believed indigenous to Australia (mainly in Western Australia and South Australia) but is also present in South Africa. Complete genome information increases our understanding of the biology of *R. toxicus*. A complete genome of *R. toxicus* isolate WAC3373 (population RT-III from Western Australia) was obtained through *de novo* assembly (HGAP3 assembly pipeline: Hierarchical Genome Assembly Process) using PacBio sequences. The finished assembly revealed one chromosome of 2,346,032 bp length (no plasmid) with a mean coverage of 99X. The GC content was 61.5%. Annotation projected a total of 2277 genes, including 2221 coding sequences (CDS) of which 1601 genes were assigned a putative function and 620 designated as hypothetical proteins. A total of 1331 genes were assigned to COG function categories. The total number of RNA genes were 51 including, 45 tRNA CDS and 6 rRNA CDS (LSU rRNA, SSU rRNA and 5S rRNA were 3117-, 1486- and 121-bp in length, respectively). Genome analyses will provide a better understanding of the genetic constitution of this high consequence bacterium. Unique regions of the genomes were identified to develop effective diagnostic tools for plant biosecurity applications.

#### **Effect of biofertilizer applications on bacterial communities in the rhizosphere of citrus affected by huanglongbing**

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Biofertilizer is increasingly used to maintain the health and productivity of citrus trees affected by huanglongbing (HLB) in Florida. Although the practice is of controversy regarding effectiveness, little is known about its non-target effects in the environment. We investigated the impact of soil application of bacterial biofertilizer agents (BFA) on rhizosphere microbial communities of HLB diseased citrus trees. Soil samples were collected at one week before applying BFA and 14 months after the first application respectively. Bacterial communities were profiled by high-throughput sequencing of 16S rDNA V3-V4 hypervariable region. Comparison of taxonomic communities only revealed marginal changes after application of BFA. The phyla Proteobacteria, Bacteroidetes Firmicutes, Actinobacteria, and Acidobacteria were most abundant in all samples, representing 95.0 to 97.0% of all assigned sequences. Analysis of the most prominent genera revealed that *Pseudomonas*, *Chitinophaga*, *Bacillus*, *Pedobacter*, *Flavobacterium*, *Bradyrhizobium*, and *Rhizobium* represented between 5% and 25% of the microbial communities in all the samples that were either treated or not treated. A general shift within the composition of the microbial communities that was independent of the application of BFA was observed after application. The results indicate that BFA application has limited effect on citrus rhizosphere bacterial communities; however, its lasting effects need to be determined.

#### **Impacts of abundance of *Candidatus Liberibacter* on the citrus phyto-microbiome**

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Understanding how the citrus microbiome is affected by (and impacts) Huanglongbing (HLB) may be critical to disease management. To test the hypothesis that phyto-microbiome composition and diversity are related to the prevalence of *Candidatus Liberibacter*, the HLB-causative agent, leaves were collected from HLB-infected Valencia trees that differed in the extent of disease symptoms and the leaf-associated microbial communities were

characterized by sequencing the V4 region of 16S rDNA. Approximately 1,900 OTUs were detected, representing 215 genera. The relative abundance of *Candidatus Liberibacter*, which ranged from 0.7 - 71.3%, induced PCA clustering and negatively correlated with alpha diversity, as indicated by Shannon ( $p < 0.001$ ) and Chao1 ( $p = 0.001$ ) indices. Positive interactions or co-occurrence ( $p < 0.05$ ) of *Candidatus Liberibacter* with other genera were detected only with *Pseudomonas* and an unclassified genus of Oxylbacteraceae. These two, in turn, had negative interactions ( $p < 0.05$ ) with 5 genera that formed a complex network of positive interactions with 39 other genera, which likely represent the “healthy” phyto-microbiome. These interactions suggest that (1) the impacts of *Candidatus Liberibacter* on the microbiome are likely mediated through its key interactions with pseudomonads and oxylbacteria and/or (2) antagonistic effects of the bacteria from the core citrus microbiome on these two groups may lessen the pathogen titer and mitigate disease progression.

#### **Quick genome sequencing of “Candidatus Liberibacter” strains by use of Enrichment-Enlargement-Next generation sequencing (EEN)**

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Members of “*Candidatus Liberibacter*” are associated with several important plant diseases such as citrus Huanglongbing (HLB) and potato zebra chip (ZC) disease. The inability to culture and the low titers in infected hosts of the bacteria have been major obstacles for research. Whole genome sequence analysis could be an effective means to study the bacteria. Taking the advancement of Next Generation Sequencing (NGS) technology, a procedure, called EEN (enrichment-enlargement-NGS), has been developed for quick genome sequencing of liberibacters. Enrichment includes increasing the DNA ratio of liberibacter to its host using alternative hosts with higher titer capacity, removal of the highly methylated plant DNA and selection of low Ct values samples; Enlargement includes increasing the enriched DNA quantity through whole DNA amplification to meet the requirement of a sequencing platform; And the NGS step includes selection of sequencing platform and development of liberibacter-specific pipelines. With EEN, >10 G of sequence data was generated from MiSeq, followed by collection of liberibacter reads using Perl scripts, de novo or/and referenced genome assembling, and sequence annotation. Thus far, seven draft genome sequences have been generated, five HLB liberibacters (China, California, and Florida) and two ZC liberibacters (California) from plant or psyllid hosts. The EEN procedure could also benefit genome research in other fastidious prokaryotes.

#### **Phenotypic effects of anti-*Candidatus Liberibacter asiaticus* antibody expression in grapefruit**

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Citrus greening, or huanglongbing (HLB), is a globally-important, fatal citrus disease caused by the gram-negative, phloem-limited bacterium “*Candidatus Liberibacter asiaticus*” (CLAs). HLB-resistant citrus germplasm is not available. This project aims to develop HLB-resistant grapefruit. Based on homology to *Xylella fastidiosa* and *E. coli*, the CLAs NodT outer membrane transporter might export factors required for virulence. The goal of this project is to block NodT-mediated virulence factor export, potentially disrupting CLAs virulence. A single-chain antibody (scFv) directed at the major predicted extracellular loop of CLAs NodT was expressed in stably transformed ‘Duncan’ grapefruit. To promote protein expression, stability and mobility in the phloem, the antibody was fused to the phloem-mobile Flowering Locus T (FT) protein. Full-length, stable FT-scFv protein was expressed in multiple transgenic lines, most of which had no phenotypic abnormalities. Thus, FT-antibody fusion protein can be expressed in citrus without deleterious effects on plant development. However, the highest expressing line exhibited precocious flowering and fruit set, presumably due to the bloom-inducing activity of the FT domain. This suggests that sufficiently high FT-scFv expression triggers flowering, which could be useful in developing rapid-flowering citrus lines to expedite breeding. The HLB resistance status of the FT-scFv grapefruit lines is now being determined.

#### **Modification of chemically defined medium XF-26 for in-vitro cultivation of *Xylella fastidiosa* and *Xylella taiwanensis* isolated in Taiwan**

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Strains of *Xylella fastidiosa* (PD) and *X. taiwanensis* (PLS) are plant pathogenic bacteria that were respectively isolated from grapevine and pear tree in Taiwan. The bacteria are nutritionally fastidious and require a complex nutrient for ex-planta growth. The Taiwan *Xylella* PD and PLS strains can grow on PD2 but not on the chemically defined XF-26 medium that was developed for in-vitro cultivation of *X. fastidiosa* strains isolated in North America. To develop a defined medium that supports growth of the Taiwan *Xylella* strains, different medium formulations modified from the original XF-26 were tested. A XF basal medium containing 20 amino acids resulted in good growth of Taiwan PD and PLS strains. By omitting amino acids one by one or in combination from the basal medium, tyrosine was found to be an essential amino acid for cultivating Taiwan PD and PLS strains, and the bacteria can grow in the XF-26 medium supplemented with tyrosine (XF-Tyr). Moreover, replacing asparagine with aspartic acid in the XF-Tyr medium also supported bacterial growth of purified PD and PLS strains or the ones isolated from diseased tissues during primary cultivation. To simplify the preparation of the chemically defined medium, the 17 amino acids in the XF-26 medium were substituted by 0.4% (w/v) casamino acids containing tyrosine, aspartic acid, and 16 other amino acids, and the modified XF-26 (mXF-26) was demonstrated to support good growth of the Taiwan-isolated *Xylella* strains.

#### **Virulence traits and disease development by *Xylella fastidiosa* are impaired in a mutant on the outer membrane protein MopB**

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Outer membrane (OM) proteins are key elements to maintain the integrity of the outer membrane, and play an important role in bacterial behavior. MopB is a major OM protein in the plant pathogen *X. fastidiosa*, which causes major losses on many economically important crops due to occlusion of the xylem network in plants. Based on DNA sequence analysis of the gene encoding MopB, the C-terminal part is a homologue of the OmpA family, and includes a conserved calcium binding motif. However the function of MopB protein in *X. fastidiosa* remains poorly understood. Here, MopB function has been studied by site-directed mutagenesis. Taking advantage of the natural competence of *X. fastidiosa*, *mopB* mutants were obtained in two different *X. fastidiosa* strains, the type strain ‘Temecula’ and the more aggressive ‘WM1-1’. *mopB* mutants in both background strains were impaired in surface attachment, biofilm formation, twitching motility and natural competence. Further analysis of the bacterial surface showed that the *mopB* mutants were impaired in pilus formation as observed by electron microscopy. Additionally, *mopB* mutants in both backgrounds showed reduced virulence as compared to WT when tested on tobacco (*Nicotiana tabacum*) plants as a host under greenhouse condition. These results suggest that MopB has an effect on pilus biogenesis and is important for the virulence of *X. fastidiosa*.

#### **Insights from comparative analyses between the genomes of two model strains of the cucurbit pathogenic bacterium *Acidovorax citrulli***

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Bacterial fruit blotch (BFB) of cucurbits, caused by *Acidovorax citrulli* (Ac), has been responsible for serious economic losses in watermelon and melon production worldwide. Ac strains are divided into two main groups based on genetic traits. While group I strains have been mainly isolated from melon, most group II strains were isolated from watermelon. The genome sequence of the group II model strain AAC00-1 is available since 2007. We recently sequenced the genome of the group I model strain M6. The M6 genome is 4.85 Mb in length, which is approximately 500 Kb shorter than that of

AAC00-1. Comparative analysis revealed this size difference is mainly explained by eight AAC00-1 genomic regions (FAs; for fragments of AAC00-1), ranging from ~35 to 120 Kb and distributed throughout the AAC00-1 genome. The FAs are absent in the M6 genome. While AAC00-1 possesses over 500 open reading frames (ORFs) that are absent in M6, only about 120 M6 ORFs are absent in AAC00-1. Coverage analyses with draft genomes of other strains support the notion that significant portions of the FAs are discriminatory between groups I and II. Moreover, GC content, effective number of codon values' and COG analyses support these FAs were introduced into group II strains by horizontal gene transfer. This study provides the first comprehensive genome comparison between the two major groups of *Ac*. Further investigation is needed to sharpen the distinctive genetic markers of the two groups.

#### **Evaluation of assembling methods on determination of whole genome sequence of *Xylella fastidiosa* blueberry bacterial leaf scorch strain**

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Blueberry bacterial leaf scorch (BBLs) disease, a threat to blueberry production in the Southern USA and elsewhere, is caused by *Xylella fastidiosa*. Efficient control of BBLs requires knowledge of the pathogen. Research is challenging because *Xylella fastidiosa* is difficult to culture. This study was to study BBLs strain based on whole genome sequence analyses. A total of 13,739,924 sequence paired reads (mean = 251 bp) were generated from a BBLs strain isolated from Georgia, USA. Draft whole genome sequences (minimum contig size = 1,000 bp) were generated by both de novo assembling (DA) and referenced assembling (RA) using CLC Genomic Workbench. The DA draft genome was 2,537K (95 contigs). Sizes (bp) of RAs varied depending on reference genome sequences: 2,449K (39 contigs) with NC\_010513 (M12, subsp. multiplex), 2,461K (70 contigs) with NC\_010577 (M23, subsp. fastidiosa), 2,467K (96 contigs) with NC\_004556 (Temecula1, subsp. fastidiosa), and 2,457K (180 contigs) with NC\_002488 (9a5c, subsp. pauca). Percentage coverages were 99.0% (M12), 97.1% (M23), 97.1% (Temecula1) and 91.7% (9a5c). ANI (average nucleotide identity) values were 99.9 (M12), 97.5 (M23), 97.6 (Temecula1) and 96.2 (9a5c). These data suggest that 1) DA alone was promising yet contig number remained high; 2) For RA, reference genome sequences had significant influence on the BBLs genome size; And 3) BBLs strain is a member of subsp. multiplex based on its similarity to strain M12.

#### **Bacterial Communities in Cultivated and Wild Cranberry Bogs**

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Transitions in microbial population structure between fallow and cultivated soils may be an important early determinant of disease-suppression of the cultivated soil. Cranberry bogs are an ideal model system for comparing native and cultivated phytobiomes because of the genetic similarity between wild and domesticated cranberry plants, and their location in the same geographic region of southeastern Massachusetts. Soil samples were collected from wild and cultivated cranberry bogs over the course of several growing seasons to determine the composition of phytobiomes in native and cultivated cranberry bogs. Population structures were determined by pyro-sequencing of the V4 region of 16S rRNA genes. Paired-end reads were assembled using PANDAseq, and analyzed using the QIIME software package to call operational taxonomic units and to calculate richness and diversity indices. Bacterial communities in both cultivated and wild bogs had different relative abundances, but were composed of five dominant phyla: *Proteobacteria*, *Acidobacteria*, *Bacteroidetes*, *Verrucomicrobia* and *Actinobacteria*. Principal Components Analysis based on soil bacterial populations among sampled sites indicate that sampling location or soil type are more important than cultivation effects in determining bacterial community structure. These results suggest that a descriptive population structure is associated with each bog type, independent of cultivation status, season, or sampling year.

#### **Host range potential of bacterial soft rot pathogens isolated from *Phalaenopsis* and *Oncidium* orchids**

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Bacteria from the genera *Pectobacterium* and *Dickeya* (formerly *Erwinia chrysanthemi*) are known to cause soft rot disease in many plant species. Since 2008, bacterial soft rot caused by a *Dickeya* sp. has been reported on several orchid genera in South Florida. Ten isolates (5 from *Phalaenopsis*, 5 from *Oncidium*) were identified as belonging to the *Dickeya* or *Pectobacterium* genera based on phenotypic and 16S sequence data and were cultured on nutrient agar for 24 h at 28°C. Cultures were suspended in sterile water and adjusted to a concentration of  $1 \times 10^8$ . Carrots, onions, potatoes (R. Burbank), and *Phalaenopsis* orchids were inoculated by placing a 10 µL drop of bacterial suspension directly over a wound made with a sterile pipette tip. Inoculated plant material was incubated at 28°C and evaluated for symptoms at 24, 48, 72, and 96 h. Bacteria were re-isolated from symptomatic tissue and identified based on the 16S and *Pel* genes. Orchid isolates caused soft rot symptoms in onion, potato, carrot, and *Phalaenopsis* orchids. Recently, *Dickeya* spp. and a newly proposed species, *D. solani*, have been reported to cause soft rot disease of potato tubers in other parts of the world, and there is concern that new species will end up in the US. We are continuing work on the orchid strains to determine their appropriate species designation.

#### **Bacteriophages: The over-looked component of the phyllosphere of *Xanthomonas*-diseased plants**

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Bacteriophages specifically infect and replicate within bacteria. Phages are the most numerous, diverse, and ubiquitous entities on earth, but little is known about their existence in the phytobiome, particularly the phyllosphere. Bacteria in the genus *Xanthomonas* cause foliar diseases in many plants. Our objective was to determine occurrence of phages on plants infected with *Xanthomonas*. Foliar samples from diseased *Brassica* spp., crape myrtle, *Corylus* sp., Nandina, pepper, poinsettia, *Prunus* spp., and Verbena were assayed. Diseased tissue was chopped/soaked in 1-3 ml sterile deionized water (SDW). Non-enrichment phage extraction used either filtering (0.22 µm) and/or treating with 10% chloroform. Indicator/host bacteria were isolated from the same disease sample or bacteria that were isolated previously from similar samples. Bacteria isolated from samples were identified using Biolog, ITS region, and elicitation of HR in a non-host plant. Bacteria lysed by phages represented 4 *Xanthomonas* spp. and 5 pathovars (pv). Phage titers greater than  $10^3$  pfu/ml extraction SDW were detected representing all 8 plant genera. Plaque morphologies were hazy *Xanthomonas*-disease microbiota.

#### **Comparative genomic analyses of 15 *Acidovorax* pathogens provide insights into the emergence of a new turfgrass disease and the host specificity**

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Bacterial etiolation and decline (BED), caused by *Acidovorax avenae* subsp. *avenae* (*Aaa*), is an emerging disease of creeping bentgrass on golf course putting greens. The causal agent of BED belongs to the genus *Acidovorax*, members of which can infect various hosts with strong host specificity. In this research, we aim to understand the emergence of BED, and identify genes in turf *Aaa* that define its host specificity. Eighteen *Acidovorax* draft genomes (14 turf, 3 maize, and 1 orchid pathogenic strains) were generated. Using whole genome SNP analysis, the 14 turf-pathogenic *Aaa* isolates were grouped into three clades. High levels of single nucleotide polymorphisms, gene deletions, insertions, and inversions were detected within the turf-pathogenic *Aaa* isolates, suggesting that the 2009-2011 BED outbreak was caused by a genetically diverse population. Population genomic analyses identified genes

whose sequences are conserved in turfgrass pathogenic *Aaa* in comparison to the closely related maize pathogenic *Aaa* isolates. Interestingly, many of these genes identified are located within the type III secretion system pathogenicity island, an important virulence factor of phytopathogenic bacteria. We identified homologous recombination as the potential cause of the gene conservation observed in the T3SS pathogenicity island. Our results suggested a strong host selection during the evolution of the turf-pathogenic *Aaa*.

#### **Epiphytic survival of *Pantoea ananatis* on Florida Pusley (*Richardia scabra* L.)**

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*Pantoea ananatis*, the causal organism of center rot of onion (*Allium cepa* L.), can survive on different weeds, but in a previous survey it was most commonly found on Florida pusley (*Richardia scabra* L.). The epiphytic survival of *P. ananatis* on Florida pusley under different temperature and moisture regimes was investigated. Weed seedlings were spray-inoculated with  $10^3$  colony forming units (CFU)/ml of rifampicin-resistant strain Pan 97-1<sup>III</sup> and incubated in a growth chamber at 15.5° or 21.1°C at 65% RH for 96 h, which represented mean environmental conditions during mid-March to mid-May in Vidalia, GA. *P. ananatis* survived for 96 h when incubated at 21.1°C with a mean population of  $3.2 \times 10^1$  CFU/g of leaf tissue. In contrast, no viable bacteria were detected after 96 h at 15.5°C. Survival of *P. ananatis* on Florida pusley was also monitored during alternating 12-h wet and dry periods, or continuous wet or dry periods for 96 h at 15.5° or 21.1°C. Compared to initial or continuous dry periods, *P. ananatis* survived significantly better with a 12/12-h wet/dry cycle or a continuous 24-h wet period at both 15.5° and 21.1°C. Unlike at 15.5 °C, *P. ananatis* populations ( $1.8 \times 10^1$  CFU/g) survived for 96 hours post inoculation at 21.1 °C under a 12-h of dry/12-h wet cycle. These results demonstrate that *P. ananatis* can survive on Florida pusley leaves under conditions of 21.1°C and prolonged leaf wetness and may potentially serve as a source of inoculum to onion.

#### **Further evidence for host preference among *Acidovorax citrulli* strains based on a detached melon fruit assay**

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Bacterial fruit blotch (BFB), caused by *Acidovorax citrulli*, is a serious threat to cucurbit crop production. *A. citrulli* strains can be divided into two genetically distinct groups with group I strains being associated with a range of cucurbits and group II strains being associated with watermelon. Despite this, seedling pathogenicity assays for *A. citrulli* lack the sensitivity to distinguish the groups. In this study, we developed an immature, detached melon (cv. Joaquin Gold) fruit assay that clearly distinguished the two *A. citrulli* groups. We observed that four group I strains induced typical water-soaked lesions on melon fruit rind tissue 7 to 10 days after pin-prick inoculation. In contrast, four group II strains did not induce symptoms on detached melon fruits. Additionally, a type III secretion (T3S) system mutant of the group I strain, M6 (M6<sup>hrcV</sup>), failed to induce watersoaking in the same assay. Based on these observations, we conclude that group I *A. citrulli* strains have the capacity to infect immature melon cv. Joaquin Gold fruit, while group II strains do not. The difference in the ability of group I and group II *A. citrulli* strains to induce watersoaking on immature melon fruit is determined by T3S effectors. Further investigation using this detached melon fruit assay will help to identify the *A. citrulli* T3S effectors responsible for this differential pathogenicity phenotype.

#### **Characterization and Management of *Ralstonia solanacearum* Causing Bacterial Wilt of Tomato in Louisiana**

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*Ralstonia solanacearum* (Rs), the causal agent of bacterial wilt of tomato can cause severe economic losses to tomato growers in Louisiana. Traditional management tactics such as soil fumigation and crop rotation are ineffective. Resistant cultivars lack durability and have undesirable horticultural traits. For these reasons, many producers in Louisiana have abandoned their fields for tomato production. Although *R. solanacearum* is endemic in Louisiana, pathogen populations have not been characterized. Tomato samples with bacterial wilt symptoms were collected from three parishes in 2015. Bacterial wilt was confirmed using commercial Rs-specific immunostrips and the bacterial streaming test. Fifteen isolates were recovered from four fields and a greenhouse and all belonged to phylotype II. Thirty-three percent of the strains were characterized as biovar 1 and 20% as biovar 3. The remaining strains did not conform to a published biovar pattern. None of the isolates were Race 3 biovar 2 based on results from R3bv2-specific PCR. Grafting with resistant rootstock varieties is being explored as a sustainable management strategy. Louisiana tomato growers were surveyed for their willingness to use grafted plants with Rs resistance. Sixty-six percent of the growers indicated that they would be willing to use Rs-resistant grafted plants but ranked not knowing how to graft and the cost of grafted plants as the top two factors that would hinder their adoption of this strategy.

#### **Development of green fluorescent protein-expressing *Xanthomonas gardneri* strains to study tomato infection processes**

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Bacterial leaf spot (BLS) is a serious disease of tomato, causing millions of dollars in losses worldwide. While BLS is associated with several species of *Xanthomonas*, *X. gardneri* is the predominant species in Ohio where it causes severe damage to processing and fresh market tomatoes. To improve our understanding of BLS disease development, two green fluorescent protein (GFP)-labeled *X. gardneri* strains were developed to study colonization *in planta*. Wild-type *X. gardneri* strains SM605-2011 and SM775-2012 were isolated from tomatoes produced in Ohio. Each strain was transformed by electroporation utilizing a GFP-expressing kanamycin/tetracycline-resistant plasmid, CGT46339. Overall, transformation efficiency for this system was low, approaching  $10^2$  CFU/ $\mu$ g DNA. Two GFP-expressing transformants, SM605-2011gfp and SM775-2012gfp, were evaluated against their wild-type counterparts for growth *in vitro*. No significant differences were observed based on optical density measurements (Permutation test,  $p > 0.05$ ), and both reached the stationary phase at 1.8 OD<sub>600</sub> and  $10^9$  CFU/ml. Stability of the GFP marker and virulence of the transformed strains will be determined. These strains will be used to study leaf, fruit and seed infection using confocal microscopy (Leica TCS SP5). The transformed strains will be a powerful tool to visualize infection processes and provide critical information for disease management.

#### **Improvement of the environmental DNA extraction method for profiling the culturable microbiome of bacterial wilt-conductive and non-conductive soils**

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The culturable microbiome profile of the bacterial wilt-conductive and nonconductive soils were compared using the PCR-DGGE profile of the V4-V5 hypervariable region of the 16S rRNA, and using the high throughput sequencing (HTS) analysis of the V1-V3 region of the 16S rRNA. PCR-DGGE results showed DNA fingerprints unique only to either the conductive or the nonconductive soils, which was confirmed by HTS analysis with 63 culturable taxa unique only to the nonconductive soil compared to 94 in the conductive soil. Exclusively cultured taxa in the nonconductive soil belong to the Phylum Planctomycete, Nitrospirae and Gemmatimonadetes while those enriched in the conductive soil belong to the Phylum Verrucomicrobia, Alphaproteobacteria and Gammaproteobacteria. While studying the culturable microbiome of the two soil types, we have improved a previous DNA extraction method to generate high quality DNA used in this study. The modified method needs no further purification steps, yields more DNA which are unsheread and with high molecular weight, with low humic acid content, and can extract DNA from bacteria of different cell wall compositions. The microbiome was cultured using low nutrient medium and with an improved DNA isolation technique we were able to capture some taxa previously

reported uncultured or were recalcitrant to culturing under traditional laboratory methods such as *Nitrospira calida*, *Planctomyces* sp., and several genera belonging to the classes Gemm-3 and Gemm-5.

### Introduction and seed transmission of *Fusarium proliferatum* in the field

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*Fusarium proliferatum* (Fp) is a seed-borne fungus in maize seed. It has a broad host range with worldwide distribution, and it is a prolific producer of mycotoxins. The introduction of this seed-borne organism into new environments can result in geographic range expansion of undesirable populations. The potential introduction of Fp via infected seed planted into a new environment and subsequent dispersal from that site via seed transmission was studied. Genotyping-by-sequencing (GBS) was used to identify unique polymorphisms in *Fusarium* species isolated from two maize genotypes. The polymorphisms were used to design primer sets to accurately detect specific isolates of Fp by end-point PCR. Extensive exclusion panels were performed to validate the accuracy of the primer sets and two Fp isolates with unique GBS loci from two maize hybrids were identified. In field experiments, 2 plots each containing 6 subplots (3 of each hybrid) were established (completely randomized design). At harvest, 20 random maize ears were collected from each subplot, and 6 ears/subplot were used. 50 kernels/ear were sampled, 1855 isolates collected, and DNA extracted; 817 isolates were identified as Fp and 751 as *F. verticillioides*. 23 isolates were identified as the unique GBS locus isolates thus indicating that transmission from the planted seed to the next generation of seed occurred. AFLP fingerprinting will confirm their identity as the original isolates that were characterized with GBS.

### Genetic mapping of pathogenesis in the northern corn leaf blight fungus

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*Setosphaeria turcica* (= *Exserohilum turcicum*) causes northern leaf blight (NLB) of maize (*Zea mays*). Identification of genes associated with pathogenesis/virulence suggests possible targets for control, generates molecular tools to characterize populations of the pathogen, and provides valuable information to breeders and maize geneticists. In this study we used a forward genetics approach to i) identify fungal genes that determine race-specific reactions with two maize resistance genes, and ii) determine the genetic regions for other virulence mechanisms in the pathogen. We generated a cross between a race 1 and a race 23N strain and collected 220 single-ascospore progeny. The ability of each progeny to cause disease on maize lines with or without the *Ht1* and *Ht2* genes was evaluated in greenhouse experiments. Mycelial abundance, radial growth, sporulation and melanin content were visually evaluated on artificial media. Genotyping-by-sequencing generated 2,078 single nucleotide polymorphic markers (SNPs). Using 310 high-quality SNPs we calculated a genetic map composed of 22 linkage groups spanning 1981.1 cM. Genetic mapping pinpointed regions of interest for the *avrHt1*, *avrHt2*, and the melanin biosynthetic locus, as well as quantitative trait loci for mycelial abundance, speed of growth, and sporulation. Molecular markers for the identification of fungal strains capable of causing disease in maize plants with *Ht1* and *Ht2* genes are being validated.

### Characterizing *Fusarium* species infecting corn roots in South Dakota

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There are multiple *Fusarium* spp. that are associated with root diseases on corn, *Zea mays*, in the United States. However, the importance of *Fusarium* associated with root disease of corn in South Dakota, is still unknown. In 2015, a survey of 50 corn fields from 23 counties in South Dakota, was conducted to characterize the *Fusarium* spp. associated with root diseases of corn. A total of 250 samples (5 plants per field) were collected between V1 (first leaf) to V3 (third leaf) growth stages. Root pieces from each sample were surface sterilized and placed on potato dextrose agar (PDA) for 7 to 10 days at 25°C under 12 hour fluorescent light/dark conditions. Isolates of *Fusarium* were hyphal-tipped, cultured, and then identified microscopically based on colony growth and spores produced on PDA. Representative *Fusarium* isolates were confirmed by sequencing of the translation elongation factor (EF1- $\alpha$ ) gene region. At least seven *Fusarium* spp. were identified. *Fusarium graminearum* and *F. oxysporum* were the most frequently recovered ( $\geq 20\%$ ) *Fusarium* spp. from the corn roots. In the aggressiveness study to compare *Fusarium* isolates in the greenhouse, significant differences in aggressiveness ( $P \leq 0.05$ ) was observed among the *Fusarium* spp. with the most aggressive isolates being *F. graminearum* (FG13) and *F. acuminatum* (FA8). Results from this research will help us develop disease management strategies for corn farmers in South Dakota to maximize their yield.

### Understanding sexual fertility in *Aspergillus flavus* through analysis of F1 progeny

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*Aspergillus flavus* produces aflatoxin, which is a constant threat and economic burden to corn and oil seed crops worldwide. In order to manage this problem more cost effectively, a greater understanding of *A. flavus* biology and genetics is required. One important question is how genetic information moves between strains. *A. flavus*, previously thought of as only asexual, has recently been found to undergo sexual reproduction both in laboratory crosses and in the field. During the mating process, the sclerotium, a survival structure, of one strain acts as the female parent providing both the mitochondria and a matrix for the ascocarps and progeny to grow, while a spore or propagule from a second strain fertilizes as the male. The fertility of mating pairs is highly variable and influenced by the directionality of the cross. We are examining crosses that exhibit high fertility with one direction, but low fertility when male and female parents are reversed. Genome wide polymorphism screening using double digest Restriction Associated DNA sequencing (ddRADseq) was performed for 36 progeny from each direction of two biased crosses. These data are being used to provide an understanding of genetic inheritance and recombination in *A. flavus* and serve as genetic markers for mapping genomic regions that may bias fertility. By understanding these aspects of *A. flavus* genetics, we create opportunities to utilize strain fertility in the selection of biological control agents.

### Progress on genomic analysis and taxonomy of the rice blast fungus (*Pyricularia oryzae*) and other Magnaporthales species

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The order Magnaporthales includes the rice blast fungus *Pyricularia oryzae* (= *Magnaporthe oryzae*), the take-all pathogen *Gaeumannomyces graminis*, and saprotrophs. Recent phylogenomic analyses strongly support the idea that the sexual genus *Magnaporthe* is polyphyletic and that the placement of the rice blast fungus in this genus was based on an incorrect morphological identification in the 1970's. The asexual genus *Pyricularia* is the older (1880s) and correct name for the blast fungus, which corresponds well with pathogenicity, and ecological and evolutionary features. Following the new "One Fungus One Name" nomenclature, we propose to use *Pyricularia oryzae* for the rice blast fungus, and treat *Magnaporthe oryzae* as a synonym that can continue to be included in publications as *Pyricularia oryzae* (= *Magnaporthe oryzae*). This will help to bridge the literature and knowledge gap for this important species. In January 2016, a workshop entitled "Comparative genomic approaches to understanding the evolution of Magnaporthales" was held at Rutgers University. About 30 working group members are currently analyzing genome data from selected Magnaporthales lineages to understand the evolution of pathogenicity and life-style adaptation. Specifically, the following research topics are being addressed: OrthoMCL analysis of gene families, the secretome, positive selection acting on orthologous genes, MAT, secondary metabolism, and the molecular clock for Magnaporthales.

### **Anything But Barren: Fungal Diversity and Functions in the Pine Barrens**

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Pine barrens ecosystem has acidic and nutrient-poor soils, where switchgrass and other stress tolerant species are dominant understory plants. Our results indicate that grass (Poaceae) roots in the pine barrens are one of the major reservoirs of novel fungi with 47% being undescribed species. Importantly, we observed that *Acidomelania panicicola* (Leotiomycetes), a new genus and species we described from switchgrass in the New Jersey Pine Barrens increases root hair growth of switchgrass and rice in low nutrient conditions. We also described another new genus *Pseudophialophora* (Magnaporthales, Sordariomycetes) that contains several pathogenic species. Naming and description of a number of other new fungal taxa are in progress. We compared the root fungal community between the wild switchgrass from the Pine Barrens and cultivated switchgrass in managed farms using both culture and metagenomic methods. Both methods suggest that Leotiomycetes are dominant fungi in the switchgrass roots from pine barrens soils, while Sordariomycetes are dominant in the roots growing in the rich farm soils. More experiments are being conducted to test our hypothesis that *Acidomelania* and other Leotiomycetes play a role in increasing plant tolerance to abiotic stresses (e.g., low pH, low nutrients, drought) and contribute to improved establishment in poor soils. Results from this work will facilitate ecological and evolutionary studies on root-associated fungi.

### **Improved plant-based culture media for growth and sporulation of *Cercospora janseana***

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*Cercospora janseana* is the fungus causing narrow brown leaf spot, a disease that has increased its importance in recent years in the U. S. *In vitro* studies are essential to better understand the biology of this pathogen and to develop effective strategies for management of this disease. However, the fungus grows very slowly and produces sparse conidia in common agar media. An *In vitro* study was conducted to evaluate various plant-based agar media in comparison with common agar media for their effects on radial growth and sporulation of *C. janseana*. Included in this study were extracts of dried rice leaves, stems, straw, roots and hulls; fresh rice leaf extract, dried extracts of barnyard grass leaves and sorghum leaves, clarified V8, and potato dextrose agar. All plant-based agar media were tested at four concentrations (25, 50, 100 and 200 g/L). Petri plates with various media were plated with a disc of 1-week-old actively growing fungus at the center and incubated at 28 °C with the 12:12 h (light:dark) cycle. Radial growth was measured at weekly intervals for 2 weeks and sporulation was measured at the end of 2 weeks. Radial growth and sporulation differed among various media and their concentrations. Among the various media tested, dried rice leaf extract, dried barnyard grass, fresh rice leaf extract and V8 media consistently supported highest radial growth of the fungus. V8 medium also supported the maximum production of conidia of this fungus.

### **Virulence of *Rhizoctonia solani* anastomosis groups on cotton, corn, rice and soybean**

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*Rhizoctonia solani* is an important pathogen on numerous crops. Isolates of *R. solani* were recovered from major row crops in the southern United States as part of a Rhizoctonia survey, and represented anastomosis groups (AG) 2, 4, 7, and 11. Virulence was evaluated for two isolates of each AG using stand and hypocotyl/coleoptile ratings on soybean, corn, rice and cotton using colonized millet seed inoculum placed 1-cm from seed at the time of planting (seed rot assay) or seedlings 7 days after planting (hypocotyl/coleoptile assay) in controlled environmental experiments at 25°C. Plant stands and discoloration ratings were recorded 7 days after planting or hypocotyl/coleoptile inoculation. In the seed rot assay, isolates of AG2 caused significant stand loss and brown-reddish discoloration of coleoptiles in corn. In cotton, the AG4 isolates were highly virulent causing significant stand loss compared to other AGs. In soybean, there were not significant differences in stands among isolates. In the hypocotyl assay on cotton, AG4 isolates caused significant damping-off. There were no significant differences in number of seedlings for corn, rice or soybeans. In all four crops, AG4 and AG11 isolates caused reddish brown lesions on hypocotyls or coleoptiles. This research supports the results of the soil and crop survey that crop increases the recovery of AGs of *R. solani* that are virulent on that crop.

### **Identity, characteristics, and fungicide sensitivity of isolates in the *Diaporthe* species complex associated with soybean stem disease in Minnesota**

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Late season stem diseases of soybean with symptoms of browning, canker, and top dieback are common in Minnesota. Characteristics of causal agents and factors that influence disease development are poorly understood. The objectives of this study were to: (i) assess the identity and relatedness among isolates in the *Diaporthe* spp. complex associated with both symptoms and asymptomatic infection, (ii) compare virulence among the isolates, and (iii) assess fungicide sensitivity. Isolates of *D. longicolla* (n=13) and *D. phaseolorum* var. *caulivora* (n=11) were obtained from symptomatic and asymptomatic plants across MN. The ITS and beta-tubulin genes were sequenced for all isolates and a phylogenetic tree with four branches was formed. Seedling stems of three soybean cultivars were inoculated with the isolates, and lesion length was measured to reveal significant differences in virulence among isolates. The groups in the consensus tree were associated with isolate species and virulence. No significant differences in sensitivity to the fungicides Folicure® 3.6F and Headline® were detected among isolates *in vitro*, and the mean EC50 for all isolates was 0.37 to 0.38 ug/ml. These studies reveal genotypic and virulence differences, but not differences in fungicide sensitivity, among isolates in the *Diaporthe* spp. complex from symptomatic and asymptomatic soybean stems, and suggest explanations for inconsistent disease development and avenues for disease management.

### **Standardizing water potential of salt-amended growth media at different temperatures for microbiological studies**

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Temperature and moisture profoundly influence the activity of fungi. Water potential (a measure of available water) for optimal growth of a fungi may differ with temperature. Depending on the habitat and life stage of the fungus, different components of the water potential (WP) become important. The matric component of WP dominates in soil but following plant infection, osmotic effects are important. Nutrient media amended with NaCl or KCl and polyethylene glycol (PEG) are used to study the response of fungi to osmotic and matric potentials, respectively. The advent of more precise WP measuring devices such as WP4C (Decagon Devices, Pullman, WA), using the chilled-mirror dew point technique, allowed us to standardize salt-amended potato dextrose broth with or without agar for a range of basal nutrient media from -0.2 to -9 MPa at 20°C, using NaCl and KCl for osmotic potential and PEG-8000 for matric potential. The same salt and nutrient media combinations standardized at 20°C were also measured for WP at 15, 25, 30 and 35°C. WP was extrapolated for 4 and 10°C with linear/curvilinear regression due to the temperature range limits of the instrument between 15 and 40°C. These standardized tables are more accurate than previous literature due to technological advancements and will be useful for studying the response of different life stages of fungi to osmotic and matric potential.

### **Ascospore biology of *Parastagonospora nodorum* under Norwegian field conditions**

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*P. nodorum* (syn. *Stagonospora nodorum*) is the dominant pathogen of the Norwegian leaf blotch disease complex on wheat and can cause substantial yield losses. To improve the forecasting model for leaf blotch diseases, available at our web-based pest- and disease forecasting platform (vips-landbruk.no), we studied ascospore biology of *P. nodorum*. Airborne ascospores were monitored with Burkard volumetric spore traps for three years in southeastern Norway. In the laboratory, we conducted wind tunnel studies to examine the effect of simulated rain and light conditions on ascospore discharge. We determined the mating types for 117 *P. nodorum* isolates (MAT1 and MAT2) using *MAT*-specific primers and inoculated wheat plants with opposite mating types to follow ascocarp maturation under field conditions. In the field, ascospore dispersal was not associated with rain events nor any specific day/night periodicity, but with daily relative humidity above 70%. In the laboratory, over 95% of ascospores were released during simulated rain events at 100% relative humidity, independent of light conditions. Mating type ratios of *P. nodorum* varied with location between 1:1 and 1:2 (M1:M2). Mature ascocarps formed 12 months after inoculation, corresponding to 551 degree-days (base 10°C) for the 2014/2015 season. We conclude that ascocarp formation can take an extended period of time and that daily humidity above 70% is sufficient for ascospore release in the field.

#### **Evaluation of chemotype, pathogenicity, and aggressiveness of *Fusarium graminearum* isolates of wheat and soybean**

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*Fusarium graminearum* is the causal agent of head blight (FHB) of wheat and damping off disease of soybean. *F. graminearum* can be classified as one of three chemotypes: 3-acetyldeoxynivalenol (3-ADON), 15-acetyldeoxynivalenol (15-ADON), and nivalenol (NIV). Besides causing yield loss, *F. graminearum* in wheat, reduces seed quality by contaminating grain with deoxynivalenol (DON) and NIV and can threaten animal and human health. Previously, 15-ADON was found to be the predominant *F. graminearum* chemotype in North America. Recently, however, the more aggressive 3-ADON chemotype is increasing in prevalence in Canada and the United States. Twelve total isolates of *F. graminearum* were collected in Wisconsin, Kansas, Iowa and Indiana from soybean and wheat between 2007 and 2015. Characterization of *F. graminearum* isolates were done using polymerase chain reaction (PCR) chemotyping. Thus far, all 12 isolates are the 15-ADON chemotype. Additional isolates will be collected in Wisconsin and further research will evaluate pathogenicity and aggressiveness on soybean and soft red winter wheat in Wisconsin. Results of this study will pave the way for the development of improved management options for Wisconsin farmers.

#### **Pathogenicity and Virulence of *Alternaria alternata* on 'Alamo' Switchgrass**

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*Alternaria alternata* causes foliar disease in switchgrass (*Panicum virgatum*), and was the most frequently isolated fungus from a survey of 30 commercial switchgrass seed lots. The aims of this study were to determine 1) pathogenicity and virulence of *A. alternata* isolates recovered from commercial switchgrass seed, and 2) the impact of isolates on seed germination, plant health, and biomass. Tests were arranged in a completely randomized design and data were analyzed with mixed model ANOVA and F-LSD (SAS 9.4). Six isolates were screened for pathogenicity with a detached leaf assay using 'Alamo' switchgrass. All *A. alternata* isolates were pathogenic on detached leaves ( $P < 0.05$ ), with lesion areas ranging from 8.8% to 23.3%. Isolates of *A. alternata* were inoculated onto surface-sterilized 'Alamo' seed at  $10^5$  conidia/ml. Plant stand and health, and biomass yield were measured. Foliar disease ratings of plants from seed inoculated with all isolates was significantly greater than in untreated controls ( $P < 0.0001$ ). Isolates were inoculated ( $10^5$  conidia/ml) on foliage of whole plants. All isolates caused greater foliar disease severity than untreated controls ( $P = 0.10$ ), and two isolates significantly reduced shoot fresh weight ( $P = 0.05$ ). Although *A. alternata* can cause foliar disease, isolates did not reduce plant stand, suggesting that this fungus may cause less economic losses than fungi that affect stand establishment, such as *Bipolaris* and *Fusarium* spp.

#### **Genetic characterization of the *Colletotrichum gloeosporioides* complex in apple orchards of North Carolina**

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Glomerella leaf spot caused by fungi in the *Colletotrichum gloeosporioides* (Cg) species complex is an important disease of apple (*Malus domestica*). It is associated with leaf and fruit spots, resulting in yield loss and premature defoliation of trees. While several studies have characterized the genotypic diversity of species complexes within *Colletotrichum*, little information is available on pathogen diversity on apple cultivars in North Carolina (NC). Development of sustainable management strategies requires consideration of both host and pathogen factors and information on their association with disease incidence and severity. The primary objective of this study was to examine the genetic diversity of field populations of Cg sampled from infected fruits and leaves of cultivars Gala, Golden Delicious, Pink Lady, Granny Smith, and Yellow Transparent in 15 orchards in NC. Genomic DNA was extracted from a phenotypically diverse subsample ( $n = 50$ ) of 150 isolates and sequence analysis of four independent genetic loci was performed to identify multi-locus haplotypes (MLHs). Eight distinct MLHs were detected, and three well-supported lineages corresponding to species in the Cg complex were identified. Isolates identified as MLH1 belonged to the Cg clade and were sampled from three of five cultivars. MLH2 isolates were identified as *C. fructicola*, and sampled from four of five cultivars with some overlap. The remaining MLHs were restricted to a single cultivar or isolate.

#### **Fitness ability and fungicide sensitivity of *Lambertella corni-maris* isolates from apple**

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*Lambertella corni-maris* is a newly reported postharvest decay of apple. Additional knowledge is needed to understand disease ecophysiology and develop adequate control strategies. In this study, we investigated fitness ability of three isolates in vitro i.e. growth on different media, sensitivity to pH and osmotic stress and in vivo pathogenicity on nine apple cultivars. All three isolates grow significantly faster on apple juice agar compared to potato dextrose agar whereas growth on V8 agar and malt extract agar was intermediate. Isolates grew equally at pH ranging from 3 to 7 and growth was inhibited at  $\text{pH} \geq 8$ . Significant sensitivity to osmotic stress was only observed at higher KCl concentrations (1M). *L. corni-maris* was pathogenic to all nine test apple cultivars, however, Honey Crisp, Fuji, Pixata, and Gala were significantly more susceptible compared to Cameo, Braeburn and Red Delicious. Furthermore, the sensitivity of 100 *L. corni-maris* isolates to fludioxonil, boscalid, pyraclostrobin, pyrimethanil and thiabendazole was evaluated using a mycelial growth inhibition assay. Mean  $\text{EC}_{50}$  values were 0.07, 0.84, 1.32, 2.45 and 3.68  $\mu\text{g/ml}$ , respectively. Fludioxonil and pyrimethanil were highly effective in controlling the disease on detached fruit whereas thiabendazole was the least effective. Our results indicate that *L. corni-maris* may be able to survive extreme conditions and is better controlled by postharvest rather than preharvest fungicides.

#### **Nutritional and environmental effects on conidial germination and appressorium formation of *Phyllosticta citricarpa*, the citrus black spot pathogen**

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Citrus black spot caused by *Phyllosticta citricarpa* has been present in Florida since 2010 and can reduce fruit yield and marketability. Conidial germination and appressorium formation (AF) are essential for successful infection by *P. citricarpa*, although the conditions required for this process are poorly understood. In this study, the effects of citrus juices, concentration, pH, various carbon and nitrogen sources, and environmental conditions were evaluated in vitro. All tested juices, especially Valencia ( $> 85\%$ ,  $P < 0.05$ ), favored germination and AF, whereas sterile water rarely stimulated



germination (< 1%). Juice quality analysis using Pearson's correlation test revealed a moderate relationship between the Brix/Acid ratio and germination ( $r = 0.783, P < 0.05$ ). The Valencia juice effect was concentration- and pH-dependent, and the maximum rate was reached in 1.5% juice with pH of 3.4. Most carbon, nitrogen, or complex sources did not favor germination or AF with exception of PDB, ammonium nitrate, and yeast extract. An incubation period of 18 to 24 h at 24°C were required for peak germination and AF. Further analysis of critical juice components using synthetic juice revealed sugars, salts, citric acid, and thiamine combined were most important for germination and AF (> 80%,  $P > 0.05$ ). These results provide a better understanding of fungal biology and a robust and convenient system for further applications such as screening for efficacious fungicides.

#### **Host colonization and substrate utilization by wood-colonizing Ascomycete fungi in the grapevine trunk disease complex**

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Grapevine trunk diseases cause chronic wood infections (cankers) in mixed infections within the same vine. To determine the synergistic interactions of trunk-pathogen communities and their impact on the host we are characterizing, on a pathogen-by-pathogen basis, fungal damage to woody cells and tissues. Our focus is on two taxonomically unrelated Ascomycetes: Botryosphaeria dieback pathogen *Neofusicoccum parvum* and Eutypa dieback pathogen *Eutypa lata*. After inoculating plants and identifying the host-defense compounds that accumulated in wood cankers, we tested their effects on fungal growth in vitro. Both fungi significantly degraded and utilized rutin, epicatechin, and piceid, suggesting that these fungi tolerate and possibly subvert the biochemical host-defense response. In contrast, fungal growth was significantly inhibited by lignin and gallic acid. Only *E. lata* discolored solid media amended with gallic acid, signifying phenoloxidase activity, which is consistent with its status as a soft-rot fungus. Previous work showed *E. lata* colonizes the ray parenchyma, xylem vessels and fibers, which we verified are also proliferated by *N. parvum*. The ability of these fungi to degrade wood was evaluated in planta by imaging the microscopic features of soft rot. As both fungi have similar gene families associated with cell-wall degradation and secondary metabolism, it is not surprising that they may share similar strategies for colonization and substrate utilization.

#### **Virulence of Botrytis prunorum associated with blossom blight on Japanese plum in Chile**

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Blossom Blight (BB) is an important disease that affects the productivity of stone fruits. Recently, *B. prunorum* was identified causing BB on Japanese plum (*Prunus salicina*) in Chile. Symptoms consisted on a light brown necrosis of flowers. The objective of this study was to compare the virulence exhibited by isolates *B. prunorum* and isolates of *B. cinerea* affecting plums. Virulence tests were conducted with seven isolates of *B. prunorum* on detached plum flowers and mature fruits 'Larry Ann' inoculated with a conidial suspension ( $10^6$  conidia/mL) and mycelial plugs (4 mm in diameter). Mature fruits of 'Granny Smith' apples and 'Hayward' kiwifruits were similarly inoculated. Flowers and fruits were incubated for at least 48 h at 20°C in humid chambers. Seven *B. cinerea* isolates were included in all virulence tests. In addition, the virulence of *B. prunorum* and *B. cinerea* was studied on detached bean leaves that were inoculated with a mycelial plug (4 mm) incubated for 4 d at 20°C in humid chambers. All isolates of *B. prunorum* and *B. cinerea* were pathogenic on flowers and fruits of plums as well as on apple and kiwifruit and bean leaf. All isolates of *B. prunorum* were significantly ( $P < 0.001$ ) less virulent than *B. cinerea* isolates. Differences in virulence between *B. cinerea* and *B. prunorum* can be attributed to differences in the genetic expression of virulence factors. These results suggest that *B. prunorum* plays a secondary role in the epidemiology of BB of plums.

#### **Modelling potential inoculum availability of Mycosphaerella nawae in persimmon leaf litter using Bayesian growth curves**

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Circular leaf spot, caused by *Mycosphaerella nawae*, is a serious disease of persimmon (*Diospyros kaki*) inducing necrotic lesions on leaves, defoliation and fruit drop. The disease was initially restricted to humid regions in Japan and South Korea and in 2008 it was detected in arid areas in Spain. The fungus forms pseudothecia in leaf litter during winter and ascospores are released in spring infecting susceptible leaves. Persimmon growers are advised to spray with fungicides during the period of inoculum availability, which was defined based on ascospore counts. In order to develop a model of potential inoculum availability, samples of leaf litter were collected weekly in six affected orchards from 2010 to 2015. Leaves were soaked, placed in a wind tunnel, and released ascospores were counted under the microscope. Hierarchical Bayesian methods were used to fit the dynamics of ascospore production in the leaf litter to Gompertz and logistic growth curves, including temperature and rainfall as nonlinear effects. MCMC methods were used to approximate the posterior distribution of the parameters. Results showed that accumulated degree days best described ascospore dynamics with both Gompertz and logistic curves. The predictive ability of the models was evaluated using ascospore data from two orchards not used in model development. The resulting best model was implemented in a disease warning system to schedule fungicide sprays for the control of circular leaf spot.

#### **Factors affecting germination of pseudosclerotia of Monilinia vaccinii-corymbosi on lowbush blueberry**

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In the lowbush blueberry (*Vaccinium angustifolium*) industry of Maine, substantial yield loss can occur annually from the disease, mummy berry, caused by *Monilinia vaccinii-corymbosi*, if left unmanaged. The primary objective of this study is to better understand the environmental conditions that favor the germination of pseudosclerotia. We have conducted *in vitro* incubation experiments for the past two years, in which the germination of apothecia from pseudosclerotia has been observed in response to chill-hours (time between 0-7°C). To further identify conditions affecting the germination of pseudosclerotia, we have incubated pseudosclerotia over a wide range of chill-hours (800-2380 chill-hours), under three different soil saturations (50, 60 and 75%), as well as three different post-chill temperature regimes (10, 13 and 16°C). Germination of pseudosclerotia was greater under the highest temperature we tested (16°C), and moderate soil moisture conditions (60%). The development of apothecia from pseudosclerotia was affected by chill-hours, but varied substantially between treatments from different fields. Identifying the environmental conditions favoring the development of apothecia in the lab assists in managing this pathogen in the field through the development of a predictive model for germination. We will use this model in continuing our program for forecasting mummy berry, which gives growers an opportunity to effectively manage this disease.

#### **Relationship between genetic and morphological characters of Botrytis cinerea isolates obtained of blueberry fields in southern Chile**

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*Botrytis cinerea* is the most important pathogen in blueberry crops (*Vaccinium* spp.) in southern Chile. The climate conditions of the zone and the presence of some populations fungicide resistant, increase its incidence. Due to the high morphological variability observed on Botrytis populations, the aim of this work was to characterize isolates from eight locations (Lat. 39°06'S to 41°28'S), in order to establish some relationship between morphological and genetic variability. For this, 39 monospore cultures of *B. cinerea* obtained from mature blueberry fruits were morphologically characterized about mycelia color, sporulation level, growth rate, conidia size and sclerotia production. On the other hand, genetic characterization was done by PCR detection of transposons *Boty* and *Flipper*, to determinate their genotype. In addition, coding fragments for the MS457 gene were sequenced and phylogenetic tree was built. The genetic studies revealed the presence of four genotypes, predominating Vacuma in the geographic region.

The results indicate that the presence of transposable elements does not affect the growth rate of the isolates. However, different sizes of conidia are distinguished by genotype, possessing at least one of the transposons conidia size increases, then Transposon presented conidia larger while Vacuole the smaller. On the other hand Boty produced the bigger sclerotia.

#### **Mining of abiotic and biotic factors for prediction of soybean sudden death syndrome (SDS) symptoms**

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*Fusarium virguliforme* is the causal agent of soybean sudden death syndrome (SDS) in the U.S. The pathogen infects roots initially, but foliar chlorosis and necrosis caused by a phytotoxin, do not usually appear until later in the growing season when it is too late to consider replanting. In this study, parameters measured before planting were used as indicators of SDS development later in the season. Prior to planting, soil nutrients (pH, K, Ca, Mg, Zn, Mn, Cu, Fe, and organic matter) soybean cyst nematode counts (eggs, juveniles, and adults), and *F. virguliforme* inoculum density were used to determine which variables or combination of variables helped explain SDS development throughout the growing season. Principle components analysis allowed for dimensional reduction and indicated which variables were most important for disease prediction. Disease symptoms at R5 and R6 were significantly correlated with soybean cyst nematode data and *F. virguliforme* inoculum density. Other multivariate techniques are currently being performed to allow for a better understanding of the factors that contribute to the disease development of SDS.

#### **Effects of inoculum substrates on root rot of soybean caused by *Fusarium oxysporum* and *Fusarium graminearum***

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*Fusarium oxysporum* (Fo) and *F. graminearum* (Fg) are associated with the Fusarium seedling disease and root rot complex in soybeans. Published soil infestation methods have limitations in the expression of disease. Our objective was to compare the efficacy of infested substrates for generating root rot caused by Fo and Fg and evaluate adverse effects of non-infested substrates on roots. Autoclaved millet and rice hulls infested with Fo and Fg were added to soil at a rate of 5% (by vol.). Susceptible cultivars Jack and MN1805 were evaluated 2 weeks after planting for root length and root rot using the WinRhizo image analysis software; root rot was also estimated visually on a percent scale. Cultivars did not differ in root length and root rot assessed by image analysis, but visually-assessed root rot was greater in Jack ( $P=0.008$ ). There was a significant interaction between substrate and Fusarium species for root rot assessed by both methods ( $P<0.0001$ ). Millet infested with Fg produced the greatest root rot severity (37%), followed by millet infested by Fo (24%), whereas infested rice hulls resulted in low root rot (15 and 10% for Fo and Fg, respectively). Root rot for non-inoculated substrates was less than 5%. Infested and non-infested millet reduced root length compared to rice hulls. Pathogen colonization was assessed using qPCR. Our study suggests that millet is more effective for producing seedling root rot but can cause some adverse effects on root growth.

#### **Preliminary results from Multilocus Sequence Typing of the soilborne fungal pathogen *Sclerotium rolfsii***

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*Sclerotium rolfsii* is an important soilborne fungal pathogen of hundreds of plant species worldwide, including economically important crops such as tomato and peanut in the southeastern US. Currently, molecular studies are scarce for this pathogen, leading to a lack of an experimental framework to study the biology *S. rolfsii* using genetic methods. There is, however, evidence for significant phenotypic variation among *S. rolfsii* isolates in terms of virulence, growth rate, sclerotial size, and temperature tolerance. Methods of measuring genetic variation are currently limited to ambiguous mycelial compatibility groups, and few sequence analyses using the ITS (internal transcribed spacer) rDNA. To accurately characterize genetic diversity among phenotypically variable *S. rolfsii* populations, a more elaborate and sequence-based experimental framework is required. Multilocus Sequence Typing (MLST) was chosen as a sequence-based method for cryptic species recognition and for identifying genetic diversity of *S. rolfsii* in the Southeastern US. Several housekeeping genes (LSU, RPB1, RPB2, TEF, and MCM7) that are commonly used in MLST analyses were evaluated to choose the most informative loci to characterize the population structure of *S. rolfsii*. The resulting MLST scheme will contribute to establishing a molecular framework for this important soilborne pathogen and will increase the current understanding of the biology and epidemiology of *S. rolfsii*.

#### **A real-time PCR and digital droplet PCR assay for quantification of *Polymyxa betae* in sugar beet roots**

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*Polymyxa betae* is an obligate pathogen capable of vectoring several viruses of sugar beet that can cause large losses in production. In the past, quantifying *P. betae* infection levels required time-consuming staining and visual examination using light microscopy. A new species-specific quantitative real-time PCR assay that allows for rapid quantification of *P. betae* infection levels in sugar beet roots has been developed. The assay has been designed to target the *P. betae* internal transcribed spacer (ITS) region of the genomic rDNA using sequence data from forty isolates. To achieve species-specificity, the assay primers and probe were designed against nineteen *Polymyxa graminis* ITS sequences from GenBank. In practice, the assay has demonstrated species-specificity in all fifteen *P. betae* isolates tested and has not amplified *P. graminis* isolates. Three internal plant controls, two targeting single copy nuclear genes and one targeting the ITS region of the rDNA, have been multiplexed with the pathogen assay to standardize results across samples. Further, the assay has also been optimized for digital droplet PCR, which allows for more precise quantification of minor differences in infection levels than conventional real time PCR. These tools will allow for more efficient quantification of *P. betae* infection levels in experimentation evaluating host resistance, variation in aggressiveness among isolates and virus transmission studies.

#### **Ray blight disease (*Stagonosporopsis tanacetii*) development in pyrethrum**

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Pyrethrum (*Tanacetum cinerariifolium*) is commercially cultivated in Australia for pyrethrins which are used as natural pesticides. Ray blight caused by *S. tanacetii* is considered as the most devastating fungal disease and is recognized as one of the limiting factors of pyrethrum production. The pathogen is known to infect the flowers and leaves however, little is known about the mechanism of infection and process of tissue colonisation. Therefore, this study was undertaken to identify disease development within the whole plant using traditional culturing and histopathology, and elucidate the life cycle of *S. tanacetii*. Ray blight disease development in pyrethrum plants was the result of colonisation of the parenchyma cells in the hypodermis and cortex with no infection of the vascular tissues. Pycnidia developed in severely infected tissues which developed a deep brown discoloration due to necrosis caused by intra- or inter cellular colonization. Secondary infection of upper flower stems, flower buds and flower tissues most likely occurred from pycniospores released from the pycnidia in the infected leaves and petioles and being disseminated through wind, rain or water-splash. The pathogen did not infect roots of plants with infected crown tissue. This process of infection and colonisation of pyrethrum plants provides a better understanding of the complete life/disease cycle which will lead to improved control measures through the strategic use of fungicides.

#### **Identifying Diversity Within *Corynespora cassiicola*, Cause of Target Spot of Cotton, Tomato, and Soybean in the Southeastern U.S.**

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Target spot, caused by the fungus *Corynespora cassiicola*, is a disease that is emerging on crops in the southeastern U.S., including cotton. Previous studies suggest that populations from cotton, tomato, and soybean in the U.S. are host specific and genetically differentiated based on host of origin. Although variation in aggressiveness to cotton was observed, no genetic variation was detected within populations sampled from cotton. The aim of this study was to develop markers to detect genetic variation in *C. cassiicola*. Variable microsatellite motifs with conserved flanking regions were identified in draft genomes of two isolates from cotton, an isolate from tomato, and an isolate from soybean. Thirty-one sets of primers were designed, and then tested for amplification of these four isolates. The nineteen loci that amplified well were evaluated on a panel of 15 isolates from diverse hosts of origin and geographic regions. Fourteen of the markers were robust and informative. Preliminary results showed diverse genotypes among *C. cassiicola* populations from different hosts, yet there was no genotypic variation detected within tomato or cotton isolates sampled from different cultivars, states, and years, suggesting that populations from these hosts represent distinct clonal genotypes. Determining levels of variation among isolates of *C. cassiicola* from cotton is critical for screening for cultivar resistance and identifying fungicides for management of target spot.

#### **Oxygen and an aerial state are independently required for asexual development of *Aspergillus* species**

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Asexual sporulation is one of the primary means for aerial dissemination of many plant pathogenic fungi. The generation of asexual spores usually involves dynamic changes in cellular polarity and morphogenesis (e.g. from filamentous hyphae to oval spores). While sporulation appears to be suppressed *in planta*, it is seemingly de-repressed outside of plant tissues, where it encounters an aerial environment. In this study, we identify key components that allow fungi to distinguish between *in planta* and aerial environments. We observed conidiophore development of several *Aspergillus* species in an artificial environment that mimicked the *in planta* condition, but where we could enforce either high or low oxygen conditions. This experimental design enabled us to reveal that not only oxygen but also the physical, aerial state are positive environmental factors inducing conidiogenesis in most of the aspergilli tested in this study. Transcriptional analysis combined with direct observation of conidiation-defective mutants revealed that expression of key regulatory genes for conidiophore development is indeed regulated by these environmental cues and that the production of reactive oxygen species is required for polarized cell differentiation. Our findings provide not only novel insights into how fungi respond to an aerial environment but also inform an improved model for the regulatory pathway controlling conidiophore development.

#### **Current Status and Ongoing Conservation Efforts for the Federally Endangered Species *Pityopsis ruthii***

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*Pityopsis ruthii* is an endangered species endemic to the Hiwassee and Ocoee rivers in Tennessee. Due to limited information regarding biology and genetics of *P. ruthii*, our research over the past six years have focused on conservation and recovery efforts. Using applied and molecular techniques, a basic understanding of the biology, propagation, population genetics, and diseases of *P. ruthii* has been gained. Micropropagation protocols using leaf and receptacle tissues have been developed. Clones from these experiments had a 73% survival rate one year after introduction to native habitats. Vegetative propagation of stems is highly successful. Seed germination for long-term stored seeds was low (<1%) and highly variable for freshly collected seeds across different locations. A total of 814 individuals from both rivers were analyzed using chloroplast and nuclear microsatellites and indicated higher genetic diversity than initially hypothesized. Analyses of chloroplast and nuclear microsatellites showed differences between the respective data sets, indicating presence of gene flow and population structure. Two diseases were identified from greenhouse grown plants: aerial blight, caused by *Rhizoctonia solani*, and powdery mildew, caused *Golovinomyces cichoreacearum*. Understanding the biology and genetics of *P. ruthii* will facilitate long-term conservation efforts and provide information on reintroduction and preservation of biodiversity for this endangered species.

#### **Genome sequencing of *Synchytrium endobioticum* offers insight into its carbohydrate degrading enzymes and effectors**

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*Synchytrium endobioticum* is an obligate fungal parasite causing severe and persistent potato wart disease that render potato tubers unmarketable. Therefore, quarantine measures are enforced worldwide to prevent the spread of this economically important plant disease. The first detection of potato wart in North America occurred in 1909 in Newfoundland. More recently in 2000, it was found in a field of Prince Edward Island. We sequenced and assembled the genomes of two different genotypes: *S. endobioticum* MB42 from the Netherlands and *S. endobioticum* DAOM 229326 from Canada. The genome size ranged from 20.6 to 23.5 Mb and between 8000 and 8800 genes were predicted for the respective strains. Carbohydrate degrading enzymes and effectors are being analyzed to help the development of more robust genotyping and pathotyping assays and to understand better the evolution of this early lineage of plant pathogens.

#### **Origin and consequence of myrtle rust (*Puccinia psidii*) in the New Caledonian biodiversity hotspot**

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The myrtle rust disease, caused by the fungus *Puccinia psidii*, is considered as one of the main environmental threat to plants belonging to Myrtaceae family worldwide. In New Caledonia, a biodiversity hotspot renowned for its extraordinary botanical richness and endemism, the fungus was first reported in 2013. The Myrtaceae, the principal target of *P. psidii*, holds a prominent position in the New Caledonian flora by being the most species-rich family. Taking into account the ecological importance of Myrtaceae in many ecosystems, it was agreed to establish a disease management approach to mitigate the spread and the potential long term impacts of the pathogen. In this perspective, it is essential to refine our understanding of the dynamics of the host-pathogen-environment interactions. In order to reach this goal, we have conducted field-based assessments of the host range and disease monitoring on several species in a semi-controlled environment during two years. A genotyping survey of the rust showed that a unique genotype has been established throughout the whole territory. To date, *P. psidii* was reported on at least 40 host species. The results showed a wide variation in responses to the disease within Myrtaceae and across different habitats, as previously showed in other locations where the rust occurs. Intraspecific variability was also observed among a range of susceptible species.

#### **Development of a multiplex PCR microsatellite marker set for *Raffaella lauricola*, and its potential applications**

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Laurel wilt is an emerging disease of trees in the family Lauraceae characterized by systemic wilt and rapid mortality. It is caused by the fungus *Raffaelea lauricola* and its vector, the redbay ambrosia beetle (*Xyleborus glabratus*) that was likely introduced to the southeastern United States from Asia. The origin of the US introduction and the population genetics of this devastating pathogen have not yet been characterized. To help facilitate this we developed a panel of microsatellite markers that can be multiplexed, amplify two or more loci in a single reaction, into eight PCRs. The marker set can be used to screen 11 *R. lauricola* isolates per 96 well PCR plate, eliminating the need to combine samples post amplification. The marker set reduces cost by using conventional hot start *Taq*, 15 $\mu$ l reaction volumes, multiplexing PCRs and fluorescein-12-dUTP to label PCR amplicons instead of fluorescently labeled primers. Future work will utilize the microsatellite marker set to investigate the genetic structure of *R. lauricola* within both the US and Asian populations and generate information regarding the number of introductions, possible origins of the introductions into the US and potential for development of new strains.

#### **Genome and transcriptome analysis of *Phellinus noxius*, a wood decay fungus**

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*Phellinus noxius*, which causes brown root rot disease in more than 200 tree species, has recently gained much attention in East Asia. However, little is known about the genome of *P. noxius*. In this study, four mate-pair libraries of a *P. noxius* basidiospore isolate A42 were sequenced by using Illumina platform. Genome assembly and *ab initio* gene prediction resulted in a draft genome of ~32 Mb ( $N_{50}$  = 1.55 Mb) which includes 11,025 coding sequences. Both *A* and *B* mating type loci were identified. Sequence alignment of A42 and a Japanese isolate showed high similarity in *B* locus and one of the two HD1-HD2 pairs in *A* locus. Sequence analysis of the *B* locus of 12 basidiospore isolates from a single basidiocarp showed no polymorphism, which suggests a bipolar mating system. To facilitate gene annotation and to better understand the expression profiles of genes involved in stress responses, transcriptomes under various stress conditions were examined, encompassing temperature shift and nutrient deficiency (minimal medium, carbon deficiency, nitrogen deficiency). Genes involved in nutrient metabolism, secondary metabolites biosynthesis, hydrophobin production, and stress responses were induced. A particularly up-regulated pattern was found in the glyoxyalate metabolism pathway which produces oxalate, an organic acid known to facilitate the activity of cell-wall degrading enzymes, as a byproduct. Investigations of the stress-related genes in disease development are underway.

#### **Ganoderma Species Associated with Declining or Dead Trees in the Southeastern United States**

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*Ganoderma* is a large and diverse genus of wood decay fungi that can cause a white rot of the lower bole or roots of hardwood, conifer and palm trees. The pathogenicity and decay ability within the genus is understudied. Some, such as *G. zonatum* are aggressive pathogens, and have been associated with tree failure and mortality of mature palms, while others, such as *G. applanatum s.l.*, are often saprophytic causing decay in old, weakened trees, but can also be facultative pathogens. *Ganoderma* species identification tools are needed to assist arborists with predicting tree failure when fruiting bodies of *Ganoderma* are present. Based on a preliminary survey of *Ganoderma* species found in Southeastern United States, over 100 isolates representing different taxa were collected from living tree species and dead wood across 4 states: Florida, Georgia, South Carolina and North Carolina. Taxa investigated in the survey were the *G. applanatum* complex and related species, *G. curtisii*, *G. meredithae*, *G. sessile*, *G. tsugae*, *G. tuberculosum*, *G. weberianum-subamboinens* species complex, *G. zonatum*, and *Tomophagus colossus* (syn. *G. colossus*). Wood block decay assays are underway to quantify decay ability of select species. Future studies will investigate differences in decay rates and pathogenicity, host specialization and phylogeography of the most commonly observed *Ganoderma* taxa collected in the Southeastern United States.

#### **Progress of severity of brown rust and orange rust during two sugarcane crop seasons in Florida**

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Brown rust (caused by *Puccinia melanocephala*) and orange rust (caused by *Puccinia kuehnii*) are currently the two most damaging fungal diseases of sugarcane in Florida. To determine progress of these two diseases during the crop season, brown rust susceptible cultivar CL90-4725 and orange rust susceptible cultivar CL85-1040 were planted in field experiments at Belle Glade in November 2013 and in November 2014. Intensity of pathogen sporulation and percent rust affected area were assessed on the top visible dewlap leaf (TVD) and the fourth leaf below TVD every two weeks. Brown rust symptoms were mainly observed from mid-March to mid-July in 2014 and from mid-April to mid-August in 2015. Sporulation intensity and leaf damage was highest in May-June 2014 but this period shifted to June-July in 2015. In contrast, orange rust symptoms were observed all year round, but sporulation and percent diseased leaf area peaked from mid-May to early August. Later on, disease severity decreased until October when another significant increase of orange rust occurred until the end of the year. This pattern of orange rust progress was similar during the 2015 and 2016 crop seasons. Overall, disease severity was higher for orange rust than for brown rust and orange rust impacted sugarcane growth for a longer period of time during the crop season. Conducive environmental conditions for disease progress remain to be determined but appear to be broader for orange rust than for brown rust.

#### **Host specificity of *Colletotrichum* sp. on tree tomato (*Solanum betaceum*) and mango (*Mangifera indica*) crops**

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*Colletotrichum* species are one of the main problems in crop production in Colombia. Several research efforts have been devoted to characterize the populations of this fungus. However, the phylogenetic relationships among species within this genus are still uncertain. *Colletotrichum* species have been traditionally classified using morphological traits and based on their host, despite the fact that the host range of these species is still unclear. In Colombia, the phylogenetic relationships of *Colletotrichum* species have been determined using molecular markers as well as morphological and physiological characteristics. Preliminary results have suggested host specificity among the species assessed. However, these assays were conducted by directly damaging the plant tissue. The aim of this study was to investigate host-pathogen interactions among isolates collected from tree tomato and mango from the pre-penetrating up to the colonization stages of the pathogen. Isolates were thoroughly characterized molecularly and microscopically and were subsequently inoculated onto both hosts. Strains of *C. gloeosporioides*, *C. asianum*, *C. theobromicola*, and *C. tamarilloi* were inoculated on whole-plants and detached-leaves. Pathogenicity, latent period, aggressiveness, and sporulation were recorded to evaluate the fitness of each species on the different hosts. Preliminary results indicate the presence of host preferences among the different species of *Colletotrichum* assessed.

#### **Arbuscular mycorrhizal fungal communities associated with organic and conventional onion crops in the Columbia Basin of Washington**

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Onion mycorrhizal associations were assessed in conventional vs. organic fields in the Columbia Basin of the Pacific Northwest USA in 2014. Soil was sampled from five sites in each of four conventional and five organic fields prior to planting in March. Arbuscular mycorrhizal fungi (AMF) were baited from each soil by planting seed of the onion cv. Tamara. Plants also were sampled from each field at bulb initiation (mid-June). Onion roots were stained

to quantify mycorrhizal colonization, and DNA was extracted for 454 pyrosequencing of AMF communities using 18S rDNA and Glomerales-specific primers. AMF were prevalent in all fields, with no significant differences in colonization of baited roots in soil samples from organic vs. conventional fields (mean  $\pm$  SE of  $77 \pm 4$  vs.  $75 \pm 4\%$ , respectively). Baited AMF communities from spring soil samples differed in community structure in conventional vs. organic fields (PERMANOVA Pseudo- $F = 4.08$ ,  $P = 0.02$ ), though AMF richness and evenness did not differ significantly (t-tests and Welch-Satterthwaite t-test  $P \geq 0.2$ ). AMF communities in mid-summer root samples also differed in community structure (PERMANOVA Pseudo- $F = 7.92$ ,  $P = 0.0005$ ), with greater richness (Welch-Satterthwaite t-test  $P = 0.02$ ) but similar evenness in organic vs. conventional fields. Differences in AMF communities between organic and conventional fields were attributed mostly to differences in relative abundance of *Glomus* and *Claroideoglomus* spp.

#### **Fungicide resistance and host susceptibility of *Colletotrichum orbiculare* infecting cucurbit crops in North Carolina**

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Cucurbit anthracnose, caused by the ascomycete *Colletotrichum orbiculare* syn. *lagenarium*, is a widespread fruit and foliar disease that results in yield losses across the United States. In North Carolina, favorable environmental conditions, host availability, and long growing seasons offer ample opportunity for disease development, resulting in significant economic losses. Application of effective fungicides and planting of resistant cultivars, along with appropriate cultural practices, have proven effective in reducing anthracnose. However, the appearance of new races of the pathogen and development of fungicide resistance has limited the efficacy of these control strategies. The objective of this study is to characterize *C. orbiculare* populations infecting cucurbit crops in North Carolina, determine their sensitivity to fungicides, and their virulence in watermelon cultivars. Isolates obtained from cucurbit fields in North Carolina and elsewhere for comparison, were evaluated for fungicide sensitivity in vitro with several fungicides and various concentrations. Local isolates of different races were used to evaluate host susceptibility of 24 popular cultivars of watermelon (*Citrullus lanatus*) in field and greenhouse trials. Results indicate that an integrated disease management strategy that combines effective fungicides and host tolerance will be required to control cucurbit anthracnose in North Carolina.

#### **Determining infection courts for *Fusarium oxysporum* f. sp. *niveum* leading to watermelon seed infestation**

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*Fusarium* wilt of watermelon caused by *Fusarium oxysporum* f. sp. *niveum* (FON) is a seedborne and seed transmitted disease. Seeds latently infected by the pathogen serve as a significant inoculum source and may result in serious disease in the field. It is unknown how watermelon seeds can get infected by the pathogen. To determine potential infection courts leading to watermelon seed infestation, experiments were conducted in 2013 to 2015 using different methods to inoculate watermelon plants with FON. Watermelon seeds were harvested when mature and seed infestation by FON was determined by isolation on peptone PCNB agar and real-time polymerase chain reaction (PCR) analysis. Swab-inoculation of immature fruit resulted in infestation of 18 to 54% of the seedlots under field conditions and 0.5 to 13% under greenhouse conditions when seeds were not surface sterilized before isolation. Seed infestation was detected on 0 to 15% of the seedlots when seeds were surface sterilized before isolation. Inoculation of stigma of female flowers resulted in infestation of 0 to 14% of the seedlots. Inoculation of peduncle also resulted in infestation of 0 to 10% of the seedlots in the field experiments when seeds were not surface sterilized before isolation. The results suggest that watermelon tissues including immature fruit, flowers and peduncle could be potential infection courts of FON leading to seed infestation by the pathogen.

#### **Evaluating isolate aggressiveness of *Fusarium oxysporum* f.sp. *niveum* in Florida**

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*Fusarium* wilt, caused by *Fusarium oxysporum* f. sp. *niveum*, is a major soil borne disease of watermelon with limited host resistance available. Understanding isolate aggressiveness is important for variety selection and other management strategies. Pathogenicity tests of 72 isolates collected from 10 Florida counties were conducted to examine their aggressiveness on the variety Black Diamond under greenhouse settings. Seedlings were inoculated with a conidial suspension using a root dip method. Aggressiveness was measured by the percentage of wilted plants and AUDPC for incidence, over a 4 week period after inoculation. Significant ( $P < 0.05$ ) differences between isolates were found using ANOVA. Based on wilted plants, isolates were characterized into 4 aggressiveness levels; not aggressive, weak, medium and severe (0%, 1-32%, 33-67% and 68-100% wilt respectively). It was observed that 33.3% of the isolates were not aggressive, 15.3% weak, 25.0% medium, and 26.4% severe. Quantitative diversity indices (Simpson's  $s = 0.27$  and Shannon entropy  $H' = 1.35$ ) indicate that these aggressiveness levels are highly diverse in Florida, and that no single level was dominant. Characterizing the aggressiveness is a necessary step for race determination, as well as the assessment of various integrated management strategies. Further research is presently being conducted that evaluates the efficacy of current integrated management strategies on isolates with a severe aggressiveness rating.

#### **Characterization of fungi associated with tomato leaf mold in the United States**

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Tomato leaf mold is one of the most prevalent diseases affecting high tunnel-grown tomatoes in the USA. Disease symptoms include chlorotic to necrotic lesions, defoliation and decreased yields. Previous studies focused on the fungus, *Passalora fulva* (syn: *Cladosporium fulvum*, *Fulvia fulva*) as the causal pathogen of leaf mold. Our initial preliminary data suggests that sporulation on lesions is associated with a number of *Cladosporium* spp., primarily *C. cladosporioides* and *C. pseudocladosporioides*. Leaf mold-associated fungi were collected from MN and NY by surface sterilization of symptomatic tissue and by direct plating of spores from lesions. Single spore isolates were generated and PCR amplification of internal transcribed spacer regions and alpha-actin were performed. Amplicons were sequenced and aligned with available reference species sequences obtained from GenBank using Seaview. A majority of leaf mold isolates collected fell within the *C. cladosporioides* complex. Representative isolates from each *Cladosporium* spp. did not appear to be pathogenic. The frequency with which *C. cladosporioides* and *C. pseudocladosporioides* are associated with leaf mold lesions suggests that they may be important in the epidemiology and etiology of the disease. Studies regarding the association between *C. cladosporioides*, *C. pseudocladosporioides* and leaf mold of tomatoes are ongoing and may lead to the development of unique disease management strategies in high-tunnel tomatoes.

#### **Mitochondrial and nuclear gene sequences to infer the phylogeny of Pezizomycotina (Ascomycota)**

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Pezizomycotina is the largest subphylum of Ascomycota. It includes filamentous species widespread in nature, where they live as saprotrophs, mutualists and/or parasites of animal, plants and fungi. We evaluated the phylogeny of such an important subphylum not only by analyzing the sequence of nuclear genes but also of the core mitochondrial ones, since this organelle may provide additional information. Hence, we compared the phylogenetic reconstitution provided by the concatenated nucleotide sequences of 12 core mitochondrial genes (*atp6*, *cob*, *cox1-3*, *nad1-6* and *nad4L*) and the individual and concatenated nucleotide sequences of 5 widely used nuclear genes ( *$\beta$ -tub*, *tef-1a*, *gpd*, *rpb1* and *rpb2*) of 28 Pezizomycotina species. Alignments were generated with ClustalW and automatically curated with Gblocks 0.91b. Best-fit model of evolution was selected with jModelTest

2.1.7 and data matrices were analyzed under maximum likelihood and bayesian criteria in PhyML 3.0 and MrBayes 3.2, respectively. We found that only *rpb2* and the concatenated nuclear and mitochondrial datasets clustered species in accordance with the current systematic of this subphylum, although incongruences were observed in some internal nodes. Discrepancies between the remaining datasets are indicative of the complex evolutionary histories and confirmed that the genes for an accurate phylogenetic estimation should be chosen with special care.

#### **In silico screening of genes coding for secondary metabolites in the phytopathogenic fungus *Stemphylium lycopersici***

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Secondary metabolites (SMs) are a highly diverse set of low molecular weight bioactive compounds that play different biological roles. They are dispensable when microorganism are cultivated *in vitro*, but usually play a key role in nature, providing organisms with adaptive advantages in different niches. Among them, toxins produced by phytopathogenic fungi play crucial roles in virulence and host nutrition. We hypothesized that the genome of *Stemphylium lycopersici* strain CIDEFI-216, an etiological agent of tomato gray leaf spot, contains several gene clusters that code for the synthesis of SMs, such as those involved in the biosynthesis of virulence factors. We searched by means of the bioinformatic tools Secondary Metabolite Unknown Region Finder (SMURF) and Antibiotics and Secondary Metabolite Analysis Shell (antiSMASH) enabling the ClusterFinder algorithm for potential SMs gene clusters within the draft genome sequence of the fungus. This led to the prediction of 32, 33 and 64 additional SMs gene clusters by SMURF, antiSMASH and ClusterFinder, respectively. They included polyketides synthase (PKS), non-ribosomal peptide synthase (NRPS), terpene synthase, dimethylallyl tryptophan synthase, hybrid PKS-NRPS, lanthipeptide synthase, fatty acid synthase and others gene clusters of unknown nature. This knowledge provides critical information to understand the biological bases of the tomato-*S. lycopersici* interaction.

#### **Greenhouse Evaluation of Dosage Responses of the Tomato I3 Gene to *Fusarium oxysporum* f. sp. *lycopersici* race 3**

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*Fusarium oxysporum* f. sp. *lycopersici* (FOL) causes an aggressive vascular wilt disease on tomato. The goal of this study was to evaluate increasing soil levels of FOL on tomato plants with and without the I3 gene conferring resistance to race 3 FOL isolates. Soil inoculum of a race 3 FOL isolate was produced in a corn meal:sand medium and diluted with a pasteurized field soil to levels of  $10^4$  to  $10^1$  cfu/g soil. Tomato seedlings with and without the I3 gene were transplanted to pots filled with 6 levels of infested soil, including a non-infested soil, and periodically evaluated for plant height and disease severity over 2 months. Afterwards, plants were uprooted, shoot fresh weights were measured, and roots and hypocotyls were plated onto Komada's medium. A significant difference in total biomass was observed between I3 homozygous and heterozygous plants, with I3 homozygous plants exhibiting 20% greater growth compared to susceptible plants regardless of the initial level of soil inoculum. Plants grown in non-infested soils were symptomless, exhibiting either undetectable or very low levels of FOL recovery from the hypocotyls. Heterozygous I3 plants grown in  $10^4$  cfu/g of FOL infested soil exhibited 70% hypocotyl infection, which was the highest rate of vascular infection of all treatments.

#### ***Fusarium oxysporum* f.sp. *lycopersici*: Small Chromosomes Define Friend vs Foe**

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*Fusarium* is a genus of filamentous fungi well-known for causing persistent wilt diseases on almost all cultivated crops. The *Fusarium oxysporum* Species Complex has no known sexual stage, yet is diverse and contains over 120 *formae speciales* that cause disease on different plant hosts. In 2010, Ma *et al.* sequenced *F. oxysporum* f.sp. *lycopersici* (Fol) to find virulence loci of differing GC content throughout the genome, including small, conditionally dispensable chromosomes (CDCs) that do not match known *Fusarium* sequences, contain genes with no known homologs, can convey pathogenicity when transferred from a pathogenic strain to a non-pathogenic relative—making them functionally similar to virulence plasmids in bacteria. CDCs could explain the high level of diversity observed in a microbe with no sexual recombination, and the inconsistent number and sizes of chromosomes consistently found in these plant pathogens. This project sampled over 40 sites, with and without known cases of Fol, to examine 1) the prevalence of CDCs where Fol is found, 2) the variation among CDCs and pathogen phenotypes, and 3) the variation within genetic backgrounds harboring different CDCs. This data will help determine the roles of CDCs in the context of the populations and growing systems in which they are found, to analyze the potential of previous or future horizontal chromosome transfer events under various conditions, and analyze the risk factors and effects on agricultural systems.

#### **Root knot nematode effects on metabolic profiles of susceptible and resistant grapevine rootstocks**

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Root knot nematodes (*Meloidogyne* spp.) can negatively impact newly planted and stressed vineyards. Nematode infestations also may increase grapevine susceptibility to other stresses such as water deficit or various diseases. However, little is known about direct or indirect effects of nematode feeding on grapevine physiology, or potential underlying mechanisms that impart resistance to certain rootstock cultivars. Therefore, this study assessed metabolite profiles in Cabernet Sauvignon grapevines grafted to either susceptible (O39-16) or resistant (Freedom) rootstocks established in root knot nematode-infested soil or steam-sterilized soil. Grapevines of both cultivars grown in soil infested with nematodes had reduced fructose and glucose levels compared to control grapevines grown in non-infested soil. Phenolic profiles in resistant grapevines did not differ due to exposure to nematodes, and only a few phenolic compounds increased in susceptible grapevines due to nematode feeding. Regardless of the presence of nematodes, root phenolic profiles between resistant and susceptible rootstocks were dissimilar, with different individual compounds more prevalent in one cultivar than the other. Taken together, these results demonstrated that nematode infestations altered root physiology, and that resistant grapevines expressed phenolic profiles different from susceptible grapevines.

#### **Influence of root exudates and soil on attachment of *Pasteuria penetrans* to *Meloidogyne arenaria***

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*Pasteuria penetrans* is a widely distributed endospore-forming bacterium which is a hyperparasite of *Meloidogyne* spp. Previous research showed that root exudates can reduce attachment of *P. penetrans* to *Meloidogyne arenaria*. The objective of this study was to determine if soil microorganisms modify the effect of root exudates on attachment of *P. penetrans* spores to *M. arenaria*. The following treatments were set up in both sand and clay soil: natural soil with a plant, sterilized soil with a plant, and natural soil without plant. For treatments with a plant, 1-month-old eggplant seedlings were used. All treatments were incubated in greenhouse for 3 days, and soil solutions were obtained by vacuum filtering saturated soil. Controls included phosphate-buffered saline (PBS) as well as root exudates in water. Second stage juveniles (J2) were incubated in 5 ml of different solutions for 6 h, rinsed and incubated in PBS for another 6 h with  $10^5$  *P. penetrans* spores. Compared to J2 incubated in PBS, attachment was significantly reduced when root exudates were present, with root exudates having a greater effect on attachment in sand than in clay. Spore attachment was always higher in clay than in

sand solutions. Root exudates reduced spore attachment more in sterilized soil than in natural soil. The presence of microorganism and chemical composition of different soils may interact with root exudates to affect endospore attachment.

#### **Soybean Cyst Nematodes of Ohio: Deciphering mechanisms of virulence**

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Soybean cyst nematodes (SCN; *Heterodera glycines*) are the most yield-reducing pest of soybean in North America. Resistant soybean cultivars are widely utilized for management purposes, however SCN populations are quickly adapting to overused sources of resistance. Counties across Ohio were surveyed for SCN populations and their virulence against the available sources of resistance was tested with HG Type assays. Of the populations tested to date, 96% are infective on PI88788, the most prevalent source of resistance in commercial varieties. The objective of this study was to determine how SCN populations have overcome this source of resistance. Nine SCN populations from across Ohio were selected, which vary in their HG Type and/or level of virulence. These populations were grown on both PI88788 and a susceptible cultivar, Lee 74. Female SCN were harvested 15 days after inoculation with juvenile worms, total RNA was extracted, and submitted for sequencing on the Illumina Hi-Seq 2500. Interesting expression patterns were found common among virulent populations such as putative effectors and genes related to digestion, the immune response, and detoxification. Also, populations avirulent on PI88788 share the expression of an effector not present in virulent populations, indicating that the loss of this effector may play a role in virulence to this resistance source. Further work includes cloning identified candidate genes to determine their role in this interaction.

#### **Investigating the role of maize for influence on soybean cyst nematode**

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Soybean cyst nematode (SCN; *Heterodera glycines*) has a significant impact on the soybean industry, costing \$90 million dollars annually. SCN has been managed mostly through the use of resistant cultivars and non-host crop rotations. Recently, resistance-breaking populations of SCN have made the use of SCN-resistant cultivars far less effective in controlling this nematode. Reliance on crop rotation has now become significantly more important for SCN control. However, little is known about how widely used non-host crops can impact SCN populations. Previous reports suggest that SCN numbers can be reduced during rotations with maize but the mode of action in this interaction is unknown. This study aimed to identify parental lines from the maize Nested Association Mapping population that are most effective at decreasing SCN populations through the production of allelopathic chemicals in root exudates. Field experiments were established using a simulated crop rotation scheme with maize and soybean. When exposed to developing maize seedlings, nematodes showed altered infection rates on soybean. A controlled experiment to complement field data collected showed significant differences in cyst production after pretreatment with maize lines. Soybean growers will ultimately directly benefit from this research by gaining knowledge on biochemical characteristics that can improve non-host selection and aid in further reducing SCN nematode populations in infested fields.

#### **Comparison of ITS1 versus ITS2 regions for characterizing fungal communities colonizing soybean cyst nematode**

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The development of next generation sequencing has enabled investigation of microbial communities from a wide range of environmental samples. We examined fungal communities associated with cysts of soybean cyst nematode (SCN) to understand the effects of different crop rotation practices. The primers most frequently used for fungal communities on the Illumina Miseq platform target either the rRNA internal transcribed spacer 1 region (ITS1) or the ITS2 region. In order to assess which primers are better for amplifying fungal communities in SCN cysts, we performed 300 bp paired-end sequencing on two Miseq runs using fungal ITS1f/ITS2r and 5.8SR/ITS4 primers. Cysts of the SCN were collected from a soybean-corn rotation field in Waseca, MN, and washed with 0.2% Tween™ 20. The DNA was extracted from 50 cysts using the CTAB method, and normalized for PCR reactions. After quality control, 361224 (ITS1) and 230404 (ITS2) reads were used for OTU picking in QIIME. OTUs were blasted against the UNITE database with the default setting in QIIME. About 99% of the ITS1 region sequences were classified as fungal sequences, however, 99% of the ITS2 region sequences were not fungal sequences. In addition, significant changes in the relative abundance of some taxa was observed between rotation treatments for only the ITS1 sequences. We conclude that primers targeting the fungal ITS1 region rather than ITS2 region are more suitable for characterizing fungal communities in SCN cysts.

#### **Gossypium arboreum accessions resistant to *Rotylenchulus reniformis* identified**

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Reniform nematode (*Rotylenchulus reniformis*) is a serious pest of upland cotton (*Gossypium hirsutum*) in the southeastern United States. Resistance does not exist naturally in *G. hirsutum* and no resistant cultivars are available. To identify sources of reniform nematode resistance, 225 *G. arboreum* accessions were evaluated in repeated growth chamber experiments. Root infection was measured on 3 plants 4 weeks after inoculation with 1,000 nematodes. The 16 accessions supporting the fewest infections were evaluated again in confirmation tests lasting 8 weeks. The numbers of nematodes extracted from soil and eggs extracted from roots of 5 plants were combined and totals were analyzed (ANOVA and differences of least squares means,  $P \leq 0.05$ ). Susceptible controls *G. hirsutum* cv Deltapine 16 and *G. arboreum* A<sub>2</sub>-101 had 85,999 and 46,902 reniform nematodes, respectively; the resistant control *G. arboreum* A<sub>2</sub>-190 had 1,665 reniform nematodes, and only 136 reniform nematodes remained in fallow pots. All 16 accessions tested supported smaller reniform nematode populations than the susceptible controls, ranging from 10,883 to 707 individuals. Nine accessions (A<sub>2</sub>-254, A<sub>2</sub>-272B, A<sub>2</sub>-294, A<sub>2</sub>-362, A<sub>2</sub>-456, A<sub>2</sub>-497, A<sub>2</sub>-514, A<sub>2</sub>-558, and A<sub>2</sub>-665) supported reniform nematode populations comparable to the resistant control, and accession A<sub>2</sub>-354 had fewer reniform nematodes than the resistant control.

#### **Movement of Fluopyram in Sandy Soil to Affect *Meloidogyne incognita* Motility**

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Fluopyram is nematicidal to *Meloidogyne incognita*. It has limited xylem movement, thus direct contact is important for nematode suppression; however, the effective movement of fluopyram in sandy soil is unknown. The objective of this study was to determine the movement of fluopyram from treated seed and a water dilution in sandy soil. Treatments consisted of 0.15 mg abamectin, 0.25 mg fluopyram, and 0.75 mg thiodicarb + imidacloprid treated cotton seed, while water dilutions of 25 µg abamectin and 25 µg fluopyram were used in the second experiment. Each treatment was placed on the surface of the soil column and flushed with 500 µl of water. The soil was removed after 24 h from three, 5-cm long segments and mixed with an equal volume of water. A portion of the supernatant was placed into a well, which contained 30-40 J2 in 500 µl of water. In the first experiment, a higher percentage of immotile J2 were observed from fluopyram collected in the 0-5 and 5-10 cm segments than abamectin. No nematode immotility was observed with thiodicarb or water control. In the second experiment, a higher percentage of immotile J2 were observed in the 5-10 cm segment from the water dilution of abamectin and fluopyram compared to the treated seed. No nematode immotility was observed past the 10-cm depth for either nematicide. The effective movement of fluopyram in sandy soil within 24 h was limited to the upper 10-cm of soil, which was similar to that of abamectin.

### **Root Knot Nematode (*Meloidogyne incognita*) populations on eggplant could be reduced using Marigold extracts**

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Root knot nematode diseases are gaining importance in vegetables in Pakistan. Chemical pesticides are often hazardous and are discouraged due to their non-targeted effects. In this study, amongst the several powder plant extracts (5 gram equivalent) of selected indigenous plants such as Marigold (*Tagetes erecta*), Akk (*Calotropis procera*), Kanair (*Nerium oleander*) and Neem (*Azadirachta indica*), the marigold extracts not only decreased the root galling index (55.1%) and number of knots per plant on root system (47.4%) in the susceptible eggplant variety (cluster king) but also increased the germination (28%) and plant vigor (32%) as compared to control plants harvested. Kanair (42.09 & 32.8%), Neem (37.56 & 28.5%) and Akk (31.51 & 21.69%) were also effective. These extracts were applied as seed treatment followed by drenching at an interval of ten days post emergence upto one month. Data was collected after two months and correlated scientifically.

### **Soybean cyst nematode culture collections and field populations from North Carolina and Missouri reveal high incidences of infection by viruses**

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Five viruses were previously discovered within Illinois greenhouse cultures of soybean cyst nematode (SCN; *Heterodera glycines*). In this study, the five viruses have been identified in SCN greenhouse and field populations from North Carolina (NC) and Missouri (MO). The prevalence and titers of viruses in SCN were examined using qRT-PCR in 47 greenhouse cultures and 25 field populations [20 from NC and 5 from MO]. Viral titers within SCN greenhouse cultures were similar throughout juvenile development, and the presence of viral anti-genomic RNAs within egg, second-stage juvenile (J2), and pooled J3 and J4 stages suggests active viral replication within the nematode. Overall, viruses were detected more frequently and at higher titers within greenhouse cultures versus field populations. Five greenhouse cultures harbored all five known viruses whereas in most populations a mix of viruses was detected. In contrast, three greenhouse cultures of similar descent to one another did not possess any detectable viruses and primarily differed in location of the cultures (NC versus MO). Furthermore, several of these SCN viruses were also present in the related nematodes species *Heterodera trifolii* (clover cyst) and *Heterodera schachtii* (beet cyst). If nematode infection with viruses is truly more common than first considered, the potential influence on nematode biology, pathogenicity, ecology, and control warrants continued investigation.

### **Potential of trap cropping for managing root-knot nematode**

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Trap cropping is a nematode management technique that has been tested periodically since the late 1800's. A susceptible host is planted and larvae of a sedentary parasitic nematode such as root-knot are induced to enter and establish a feeding site. Once this has occurred, and the female begins to mature, she is unable to leave the root. The plants are then destroyed before the life cycle of the nematode can be completed, trapping nematodes within the root. By itself, trap cropping is not likely to provide the same level of control as a fumigant nematicide, because not all nematodes are induced to enter the roots. However, the potential for loss of registration of fumigants for various environmental reasons is great enough that an IPM approach using two or more techniques in combination that will each provide partial control of the nematode population is warranted. Several years of field testing of trap crops with and without a biological nematicide for management of root-knot nematode on carrots demonstrated that trap cropping resulted in statistically significant increases in weight and number of marketable carrots. A wet fallow treatment, in which a trap crop was not planted, but the weeds that germinated following irrigation served to trap nematodes within the roots, followed by tillage two weeks later, also produced a statistically greater yield of marketable carrots than the untreated.

### **Cover cropping affects plant-parasitic and free-living nematodes in Michigan carrot production**

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Cover crops are often used for reducing soil erosion, retaining soil nutrients, and building organic matter. Free-living nematodes (nematodes that do not feed on plants) are well-established bioindicators and may respond to changes in soil ecology due to cover cropping. Some cover crop cultivars are also marketed for managing plant-parasitic nematodes. This study assessed the impact of common Michigan cover crops on plant-parasitic and free-living nematodes in carrot production systems. Research was conducted at 4 sites in Michigan where cover crops were grown in the fall preceding summer carrot production. Oats, 'Defender' radish, dwarf essex rape, a mixture of oats and 'Defender' radish, and a fallow control were treatments at each site. Black oats and 'Grazia' radish were also grown at sites 3 and 4 while 'Image' radish was grown only at site 4. Lesion nematode (*Pratylenchus* sp.) was present at all four sites. At sites 1, 3, and 4, lesion nematode densities were relatively small (less than 20 nematodes/100 cm<sup>3</sup> soil) and not significantly ( $P > 0.05$ ) affected by cover crops. At site 2, during carrot production, lesion nematode density was significantly ( $P < 0.05$ ) greater following 'Defender' radish than fallow or other cover crop treatments. Similarly, at site 2 during carrot production, free-living nematode densities were greater following 'Defender' radish or oats than fallow, but were not significantly affected by cover crops at the other sites.

### **Mode of action studies on the nematicide fluensulfone**

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Fluensulfone has proven efficacy in the field against a range of nematode species. The mode of action of fluensulfone is however currently unknown. Fluensulfone was found to have nematicidal activity against *C. elegans* but at concentrations  $\geq 100$ -fold greater than those reported to be effective against the PPN *Meloidogyne javanica*. Fluensulfone affected a number of *C. elegans* behaviours, including locomotion, pharyngeal pumping, egg laying and development. The pharyngeal effects suggested that fluensulfone might influence feeding behaviour in PPNs. In PPNs, the behaviour of the stylet mouth spear is critical in feeding and host invasion. The action of fluensulfone on the stylet of the PPN *Globodera pallida* was therefore investigated, to validate observations made with *C. elegans*. The pharmacology of the stylet was also investigated, using knowledge of the *C. elegans* pharynx as a guide. Fluensulfone stimulated stylet activity and blocked 5-HT-stimulated activity. If metabolic impairment is identified as the causative agent of fluensulfone nematicidal activity this must be further studied through metabolomics and other techniques.

### **Temporal analysis of oomycete communities associated with soybean and corn**

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Corn and soybean rotation is typically practiced in field crop production, with the purported benefit of reducing pathogen inoculum density. This management practice has been studied before with concentration on a single pathogen species. There is limited information on how the associated oomycete community is modulated by using different crops in the rotation. We hypothesized that the genetic background of the hosts and their different growth stages could have an effect on the associated oomycete community. In 2013-14, field trials including two soybean cultivars (cv. Sloan and



Archer) and two corn cultivars (cv. DK5261 and P0255AMXT) were planted in a randomized block design with non- and inoculated treatments. The inoculated treatment consisted of a mixed inoculum of equal parts of *Pythium irregulare*, *Py. sylvaticum*, *Py. ultimum*, *Py. heterothallicum*, *Py. oopapillum* and *Phytophthora sojae* colonized rice. Soil and root samples were collected at different growth stages and subjected to DNA extraction. Samples were sequenced for ITS 1 amplicons. The results showed a variability in community composition between the two crops, however cultivars did not significantly vary in community composition despite different genetic backgrounds. Host could play an important role in filtering the associated oomycete community, however it did not effectively change the composition, suggesting this specific rotation system might not be conducive to pathogen inoculum reduction.

#### **Survey data reveals potential interactions between soil-borne fungi and oomycetes isolated in the midwest**

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Root rot pathogens, such as *Fusarium* and *Pythium* species are frequently isolated together from damped-off soybean seedlings. Consequently, they are hypothesized to form a multi-species complex that infects soybean, however the interaction has not been extensively studied and is not well understood. The objective of this research was to identify potential interactions between fungi and oomycetes. Data from 2012 Midwest survey funded by the USDA-NIFA and USB/NCSR was used. Data comprised species recovered from seedlings collected by soybean pathologists from fields with seedling disease or a history of disease. Hierarchical clustering was used to identify groups of isolates frequently recovered together from the same field. When data were analyzed including all fungi and oomycetes, regardless of pathogenicity, six distinct clusters were identified. Within each cluster were fungal and oomycete pathogens and non-pathogens. When data were reanalyzed with only pathogenic species, four distinct clusters were present. Cluster 3 contained only *Fusarium* species, while cluster 2 contained several *Pythium* species as well as *Phytophthora sojae*, *Phytophthora sansomeana* and *F. verticillioides*. The remaining two clusters contained *Fusarium* and *Pythium* species. These data should allow soybean pathologists to identify potential interactions between specific species of soil-borne fungi and oomycetes that can be explored and used to enhance our understanding of root rot on soybean.

#### **Breadth of resistance of *Phytophthora* fruit rot resistant watermelon germplasm to *Phytophthora capsici* isolates from across United States of America**

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*Phytophthora* fruit rot of watermelon caused by *Phytophthora capsici* is particularly severe in southeastern U.S where optimal conditions for disease development are prevalent. We have developed *Phytophthora* fruit rot resistant (PFR) germplasm lines (USVL020-PFR, USVL203-PFR, USVL489-PFR and USVL782-PFR) for use in breeding programs. The breadth of resistance of these lines to 20 *P. capsici* isolates collected from various states and different crops was evaluated. Mature fruit of PFR lines or susceptible checks 'Mickylee' and 'Sugar Baby' were collected from plants grown in a field. Fruit were inoculated by placing a 7-mm agar plug from a 4-day-old colony of a given *P. capsici* isolate. Inoculated fruit were kept in a walk-in-humid chamber (>95% RH, temperature 26±2 °C) for 5 days. Ratings on lesion diameter and visible pathogen growth were then recorded. Variability in aggressiveness among the 20 isolates on checks and some PFR lines was observed. Check cultivars were susceptible to all isolates whereas PFR lines were significantly more resistant. However, PFR lines were not immune to all isolates. Significant differences in lesion size on PFR lines for some isolates was observed. In most cases very small lesions (0.8mm) formed on PFR lines under the agar plug and on the fruit surface. Our results suggest that watermelon cultivars developed using these PFR lines as resistant sources will be able to provide resistance against *Phytophthora* fruit rot across many states.

#### **The Effect of Increased Soil Fertility on Seedling Disease Development of Soybean in Ohio**

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Low or high soil fertility levels have been shown to have potential secondary impacts on root pathogens, some of which decrease yield. Fertility recommendations for soybean in Ohio are over 20 years old and one proposed scenario is that more phosphorus and potassium may be required to support the increase in yields over the past 2 decades. Therefore, the objective of this study was to evaluate the impact of higher rates of P, K, and P + K on seedling diseases caused by oomycetes in both field and greenhouse trials. Field trials were carried out at two locations in 2014 and three locations in 2015 at fields each with a known history of seedling disease. Stand counts, percent emergence, and yield were not significantly different between treatments at any of the locations. However, a greater number of *Pythium* spp. were recovered from plants with the addition of K. In greenhouse assays using soil collected from the same infested fields, final stand and root weights were significantly ( $P<0.05$ ) higher in pots without fertility treatments for one of the two locations. Further monitoring of the influence of higher rates of K at the time of planting on disease development of soil borne diseases caused by oomycetes is warranted.

#### **Cold stress at planting increase susceptibility to damping-off caused by *Pythium sylvaticum***

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*Pythium sylvaticum* is an Oomycete that causes damping-off of soybeans in Iowa, particularly when cool weather occurs at planting. Seed treatments are applied to over 90 percent of soybean seed in Iowa to protect germinating seedlings and consequently plant stand. The objectives of this experiment were to (i) evaluate the effect cold stress at different times after planting, and (ii) the effect of seed treatments on damping off. Soybean seed was treated with Intego Suite, Intego Suite + mandestrobin or left untreated and planted into 8 oz cups inoculated with *P. sylvaticum* or non-inoculated. The cups were placed in growth chamber and subjected to 96h of cold stress (4°C) at 0h or 24h after planting. A significant three-way interaction was detected between cold stress, inoculation and seed treatment ( $P<0.0001$ ). In general, emergence of untreated seeds was reduced more than 70% when subjected to periods of cold stress ( $P<0.05$ ). Emergence was improved with seed treatments ( $P<0.0001$ ). These data indicate that periods of cold stress at planting increases the susceptibility of soybean to damping-off caused by *P. sylvaticum* and that seed treatments reduce damping-off under these adverse conditions.

#### **Pathogenicity and Virulence of Oomycete Species on Common Bean**

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Common bean is often affected by soilborne oomycetes that cause seed and seedling rot. A study was conducted to determine the pathogenicity and virulence of 24 *Pythium* spp. and *Phytophthora* aff. *vexans* on two common bean (*Phaseolus vulgaris*) cultivars, 'Red Hawk' kidney bean and 'Zorro' black bean from Andean and Meso-American gene pools, respectively. A seedling assay was conducted at 20°C in a growth chamber. Oomycete inocula were added to the root zone at the time of planting, and seedlings were harvested after 12 days. Root length, root area, root dry weight, and emergence were quantified. Seedling assay results indicated that 12 species caused significant root length and root area reductions from the control. Similarly, 11 of the same oomycete species caused significant reductions in root dry weight. Four *Pythium* spp. and *Phytophthora* aff. *vexans* caused significantly lower emergence than the control and were considered to be seed rot pathogens. An *in vitro* seed assay was conducted at 20°C and 26°C, in which disease

ratings were assessed. In this assay, 13 species caused disease severity that was significantly higher than the control. Moreover, it was observed that the virulence of certain oomycete species varied depending temperature and host gene pool. Findings help elucidate the oomycete species that are most aggressive and identify factors that may influence oomycete species virulence on common bean.

#### **Putative sucrose esters from *Petunia x hybrida* may contribute to this host's reduced susceptibility to *Phytophthora infestans***

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*Petunia*s have been established as a less susceptible host of *P. infestans*, but the reasons for this have not been elucidated. We have reported that *petunia* leaf extracts contain a potent zoosporicide. Previous biochemical analyses of crude leaf extracts suggested the presence of sucrose esters, a class of compounds known to be produced by the trichomes of *Petunia* and *Nicotiana* species. Subsequent experiments with commercially available sucrose esters have revealed that sucrose monolaurate (SM), an amphiphilic sucrose monoester known to interact with cell membranes, exhibits zoospore lysing activity. The goal of our current work is to confirm the presence of and identify the chemical structure of the putative sucrose esters present in *petunia* leaf extracts. Samples were purified using hydrophilic-lipophilic balanced cartridges and an established protocol for the purification of sucrose esters from *Nicotiana tabacum*. The purified samples were analyzed, along with SM as a control, via LCMS-ELSD, <sup>1</sup>H and <sup>13</sup>C NMR, and TLC. The data shows a lack of monoesters, excluding SM as the bioactive component, but supports the presence of several known sucrose di- and polyesters. Analyses are ongoing to deduce the specific chemical structures of these sucrose esters. Results from this work will bring us one step closer to understanding an aspect of the *petunia*-late blight interaction that has received little attention.

#### **Identification and characterization of potential oomycete pathogens from the University Agricultural Laboratory in Fresno, California**

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The University Agricultural Laboratory (UAL) at California State University, Fresno is a 1000-acre farm that grows a wide range of fruit, nut, vegetable, and field crops. Many of the crops grown on the UAL are negatively impacted by oomycetes; however, identification of pathogenic species has not been determined. Therefore, the goal of this research was to identify and characterize potential sources of oomycete pathogens from soil and irrigation reservoirs on the UAL. Oomycetes were baited over a 5-day period using pears or cucumbers as bait. Symptomatic fruit tissue was excised and plated on the oomycete selective medium PARP. Isolates were then characterized using morphology and DNA sequencing of the internal transcribed spacer region. Forty-four isolates of *Pythium* spp., 6 isolates of *Phytophthora* spp., 4 isolates of *Phytophthora* spp., and 1 isolate of a *Pythiogeton* sp. were identified. *Pythium* spp. (86%), *Phytophthora* spp. (11%), and *Phytophthora* spp. (3%) were identified from the irrigation reservoir. The most common species isolated from irrigation water were *Pythium oopapillum* (54%) and *Pythium dissotocum* (25%). Of the isolates baited from soil 75% were *Pythium* spp., while 13%, 8%, and 4% were *Phytophthora* spp., *Phytophthora* spp., and a *Pythiogeton* sp. respectively. These results suggest that the UAL has a diverse number of potential oomycete pathogens and that irrigation reservoirs may act as a source for potential inoculum in farming operations.

#### **Development of a split-root technique for canola (*Brassica napus* L.)**

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Canola (*Brassica napus*) is an important oilseed crop in Canada. A split-root system was developed for studies of host-pathogen interaction with root pathogens. Canola seed was germinated on moistened filter paper within pouches. After 7 days, the root tip of each seedling was clipped back to stimulate secondary branching. Each seedling was then carefully planted into soilless mix and allowed to grow for 18 days. When uprooted, each seedling had a pair of secondary roots. One root of each pair was then potted into each side of a specially modified plant pot for subsequent growth and assessment. The modified pots were created by placing two small plastic bags filled with potting mix into a square pot to create two equal but independent sections of soil per pot. A bamboo support was inserted between the bags. The roots were placed into each bag and covered with soil. The young seedling was loosely tied to the support, then sand was added to cover the soil and support the seedling. This resulted in almost 100% survival of the treated seedlings. As an initial proof of concept, seedling roots on one side of each pot were inoculated with resting spores of *Plasmodiophora brassicae* 7 days after potting and visually assessed for clubroot symptoms at 42 days after inoculation. Clubroot symptoms developed on the inoculated side but not on the control.

#### **Estimates of viable resting spores of *Plasmodiophora brassicae* using propidium monoazide and qPCR**

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*Plasmodiophora brassicae* [clubroot of *Brassica* spp.] persists in soil as long-lived resting spores. Quantitative polymerase chain reaction (qPCR) estimates of spore concentration do not distinguish between viable and non-viable spores. Propidium monoazide (PMA) penetrates non-viable cells and binds to DNA when exposed to high-intensity light, and so inhibits amplification with qPCR. The impact of pre-treating resting spores with PMA and light prior to qPCR (PMA-PCR) was assessed. Spore suspensions from 6-wk-old (immature) and 9-wk-old (mature) clubs were heat-treated, and concentrations of spores were estimated using qPCR and PMA-PCR. Spore viability was assessed using bioassays on canola seedlings. Prior to heat treatment, comparison of estimates from qPCR and PMA-PCR indicated that all mature resting spores were viable, but  $\geq 74\%$  of immature spores were not viable. This was consistent with substantially higher clubroot severity in plants inoculated with mature resting spores than immature spores. Heat treatment produced little or no change in estimates of mature spores with qPCR, but substantially reduced both estimates of spore concentration from PMA-PCR and clubroot severity in bioassays. For immature spores, PMA-PCR did not consistently demonstrate a heat treatment effect. We conclude that PMA allows for estimation of viable resting spores using qPCR.

#### **Phenotypic variation between two clades of *Phytophthora cinnamomi* isolated from avocado fields in California**

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*Phytophthora cinnamomi* (*Pc*) is the causal agent of Phytophthora root rot (PRR), the most serious disease of avocado worldwide. Pagliaccia *et al.* (2013) first assessed the genetic diversity of this pathogen in California and identified 16 genotypes corresponding to two distinct clades of A2 mating type isolates. Additionally, differences in virulence were observed between these two clades after infecting avocado rootstocks with different levels of tolerance to *Pc*. To reveal additional phenotypic differences in these specific genotypes, three representative isolates from clade I and clade II were characterized for: i) mycelial growth rate at different temperatures, ii) zoospore production, iii) zoospore germination, and iv) fungicide sensitivity. More virulent *Pc* isolates of clade II showed slower growth but an earlier release of zoospores. The molecular interaction between these *Pc* isolates and their hosts are being studied using lupin and avocado plants. *Pc* nuclear and mitochondrial genes will be used to study the genetic relationship among isolates from these two clades. Our data suggests that the isolates representing clade II differ in most of the phenotypic traits assessed. Fungicide efficacy among these isolates is being tested under greenhouse conditions using avocado seedlings. This work represents an integrated approach to study the molecular interaction of *P. cinnamomi* with its host and provide practical solutions to manage this pathogen in the field.

### **Phylogenetic relationships among Philippine isolates of *Peronosclerospora* based on sequence analysis of multiple mitochondrial loci**

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Downy mildews cause substantial yield losses of sugarcane and corn in the Philippines with several species of *Peronosclerospora* (*P. sacchari*, *P. spontanea*, *P. miscanthi*, and *P. philippinensis*) commonly reported. Due to the obligate nature of the pathogen only spore morphology and host genus have been used as the bases of species identification. However, these parameters are highly effected by environmental conditions. Therefore, molecular tools are needed for investigating pathogen diversity and taxonomy. Primers have been developed for amplification of three mitochondrial loci from fifty-four isolates of *Peronosclerospora* recovered primarily from sugarcane representing geographically diverse regions in the Philippines. Limited sequence divergence of the mitochondrial loci was observed among these isolates. Additional isolates recovered from corn are being sequenced and added to the analysis to evaluate the taxonomy and phylogenetic relationships among corn and sugarcane downy mildews. Studies involving a broad sampling of the pathogen from other grasses in the Philippines and neighboring Asian countries are being conducted to assess the diversity and identify species of tropical graminicolous downy mildews. To facilitate rapid pathogen identification, species-specific molecular diagnostic assays targeting mitochondrial loci are under development. This is especially important for detection of the USDA-APHIS Select Agent *P. philippinensis*.

### **Identification of conserved transcriptome responses to propagative plant viruses in insect vectors**

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The plant *Nucleorhabdovirus*, *Maize mosaic virus* (MMV), is vectored by the corn planthopper, *Peregrinus maidis*. Previously, we conducted an RNA-Seq project to identify differentially expressed (DE) transcripts in adults during infection with MMV. We identified a total of 144 transcripts, with 77 genes upregulated and 67 genes downregulated. To identify conserved transcriptome responses in vectors to propagative viruses, we compared the DE transcripts in our system to two other systems: white backed planthoppers, *Sogatella furcifera*, exposed to *Southern rice black-streaked dwarf virus* (SRBSDV, *Fijivirus*), and the black-faced leafhopper, *Graminella nigrifrons*, infected with *Maize fine streak virus* (MFSV, *Nucleorhabdovirus*). In a tblastx analysis with a cut-off value of 10<sup>-3</sup>, we identified DE transcripts that are shared between systems. Twenty three transcripts were shared between all three systems. Two transcripts were unique to *P. maidis* and *G. nigrifrons* and 38 transcripts were unique to *P. maidis* and *S. furcifera*. Protein binding was the largest category of the 144 *P. maidis* transcripts (19), 10 were shared in all three systems. Functional characterization is underway on transcripts potentially involved in viral replication, intercellular movement and defense. The responsive genes identified in these vectors of propagative viruses may represent a conserved anti-viral strategy and could be targets for resistance against a diverse range of viruses.

### **An optimized multiplex detection protocol captures divergent isolates of yellow dwarf virus species in Kansas wheat**

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Barley yellow dwarf (BYD) is a complex aphid-transmitted virus disease that can be caused by multiple yellow dwarf virus (YDV) species classified in the family *Luteoviridae*. Previous research revealed that *Barley yellow dwarf virus-PAS* (PAS) is prevalent in KS. However, currently available multiplex RT-PCR detection assays designed for a subset of YDVs do not include PAS, and primers we designed to the reference sequence of PAS (NC\_002160) did not detect all KS isolates. The objectives of this study were to i) sequence two KS field isolates of PAS and one of PAV to enable primer design, and ii) to modify and optimize a multiplex RT-PCR assay to detect six YDV species, including divergent isolates of KS PAS. To test the new assay, 101 archived single-plant leaf samples obtained from a wheat virus survey were tested to determine YDV species composition. We found that PAS was the predominant isolate detected with 59% incidence followed by PAV with 57%; mixed infections of PAS and PAV (30%) were common. Single infections for PAS and PAV (23% each) were also found. The other YDVs were only detected in mixed infections of PAS and/or PAV in 12%, or less, of the samples. These include *Cereal yellow dwarf virus-RPV*, BYDV-SGV, BYDV-MAV, and a variant of *Maize yellow dwarf virus-RMV* first identified in MT. This new protocol will provide a tool for examining the association between BYD disease development and YDV species composition in Kansas wheat fields.

### **Impact of downy brome (*Bromus tectorum*) and volunteer wheat on risk of winter wheat infection by *Wheat streak mosaic virus***

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Wheat streak mosaic is caused by *Wheat streak mosaic virus* (WSMV) which is vectored by the wheat curl mite (WCM, *Aceria tosichella* Keifer). The mite and virus can survive on multiple grass species that act as reservoirs for WSMV. Understanding the effect of alternative hosts on risk of WCM infestation and WSMV infection in newly emerged winter wheat crops is crucial for disease management. We assessed WCM immigration onto trap plants and the influence of alternative hosts such as downy brome (*Bromus tectorum* L), and volunteer wheat on WCM infestation and WSMV infection of wheat. Wheat trap plants were monitored weekly in the fall over three years to assess the relationship among patterns of WCM infestation and WSMV infection with cumulative growing degrees days (GDD) of spring wheat, daily mean temperature with respect to alternative hosts. The relationship among GDD on WCM infestation and WSMV infection across alternative hosts was non-linear. Mite infestation and virus infection was positively correlated with daily mean temperature ( $P < 0.001$ ) in the fall. The risk of WCM infestation and WSMV infection on trap plants from downy brome and volunteer wheat plots was higher during the late fall when compared to the bare ground control plots ( $P < 0.05$ ). Overall, daily mean temperature is a better predictor of WSMV risk and the Wheat streak mosaic infection on emerging crop increases with the presence of downy brome and volunteer wheat species during the fall.

### **A full genome cDNA clone of Cherry rusty mottle associated virus induces disease in sweet cherry**

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Cherry rusty mottle-associated virus (CRMaV), a member of the family *Betaflexiviridae*, is associated with cherry rusty mottle disease (CRMD). To date, fulfillment of Koch's postulates for this virus has yet to be demonstrated. A full genome of CRMaV isolate 98CI194 was cloned into a modified pORE-E4 *Agrobacterium* binary vector between the ENT2CUP2 promoter and the *Nos-t* terminator. Leaf agroinfiltration with the binary vector carrying ENT2CUP2-CRMaV failed to infect *Nicotiana benthamiana*, *N. occidentalis* 37B, and *N. glutinosa* whereas agro-inoculation using either the bark flap technique or root dipping resulted in the induction of CRMD symptoms in Krymsk 6 virus-tested cherry rootstock. Further confirmation of infection was verified by RT-PCR detection of CRMaV amplicon and by positive reaction of symptomatic leaves in ELISA and Western blotting using an antibody against the coat protein of CRMaV 98CI194. Finally, bark patch inoculation using lignified wood from one of the CRMaV positive, agroinoculated, Krymsk 6 trees resulted in CRMD symptoms on virus-tested sweet cherry seedling rootstock *Prunus avium* 'Mazzard'. These results provide conclusive evidence of the etiology of CRMD to be caused by CRMaV. This infectious clone provides a tool for future studies of genome expression and pathogenesis of CRMaV. Moreover, the construction of this infectious clone opens a path forward to pursue etiological studies of other stone fruit virus-like diseases.

### **Comparative analyses of world-wide cucumber green mottle mosaic virus (CGMMV) isolates**

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CGMMV is a +ssRNA *Tobamovirus* that infects cucurbit crops. It is capable of contaminating seeds inside the seed coat, making it a problem with the increased globalization of seed trade. 2013 was the first report of this virus in North America, which prompted federal and state responses and quarantine procedures where it was detected. Subsequent finds in 2014 and 2015 occurred in California, suggesting this is a recurring problem with global seed movement. Our efforts focus on better detection methods in seed. Currently, industry standard for detection is ELISA, which relies on antibody recognition of coat protein. Industry participants have described isolates that are not detected with available ELISA kits, but are identified with molecular assays. We are working to improve detection methods for CGMMV by serological and molecular methods. To support those efforts we need a better understanding of the diversity of CGMMV isolates. We previously sequenced two California isolates and found them to share 90% sequence homology. GenBank has 197 accessions for CGMMV, of which 42 are full genome sequences. Of these, the majority are Asian in origin. Given that CGMMV was originally described in England and is endemic to Europe and the Middle East, we worked with collaborators to amass a collection of 35 isolates from regions where CGMMV is endemic. We used enriched virion samples for next generation sequencing, and bioinformatics to analyze the sequence information.

#### **The viroome of *Rhizoctonia solani* and its effect in fungal pathogenicity**

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*Rhizoctonia solani* consists of at least 14 genetically distinct anastomosis groups (AG). Mycoviruses have been shown to play an important role in fungal epidemiology and pathogenicity. The goals of this research are to understand mycovirus epidemiology in *Rhizoctonia solani* on important row crops in Arkansas and their potential to influence pathogenicity. Mycoviruses have been extracted from 190 geotagged isolates from five different fields using cellulose affinity chromatography. The cDNA from double-stranded RNA enriched material was sequenced and sequences analyzed to determine whether they belong to known or undescribed viruses. Detection primers were designed for each virus and then used in all geotagged isolates. Our results are consistent with previous research indicating that mycoviruses are commonly found in isolates of *R. solani*. More than 20 new viruses, belonging to at least three different virus families have been discovered. Future experiments will determine the effects of mycovirus presence on growth and virulence of the fungus, and to determine rates of horizontal transmission. Spatial analysis will determine whether interactions exist between *Rhizoctonia* spp., AG, and mycovirus distribution in fields, as well as crop and soil properties.

#### **Studies on Groundnut rosette disease and implications of the newly reported *Groundnut ringspot virus* for groundnut production in Ghana**

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Groundnut rosette is a devastating disease of groundnut (peanut) and presents a major constraint to crop production. Twelve cultivars of groundnut were screened in field trials for resistance to Groundnut rosette disease (GRD), caused by infection with *Groundnut rosette assistor virus* (GRAV), *Groundnut rosette virus* (GRV) and its satellite RNA. GRAV infection rates were higher in the wet (13.9–100%) than the dry (11.8–61.8%) season. Four local varieties were rated as resistant along with three of six improved varieties previously regarded as possessing resistance to GRD. These resistant cultivars could be used in the interim management of the disease in the field and further exploited in breeding programmes for the development of virus-resistance. *Groundnut ringspot virus* (GRSV)-like symptoms were observed within these trials. The identity of the virus was confirmed by ELISA, RT-PCR and amplicon sequencing, and represents a first record of GRSV for Ghana. GRSV was found in most cases in mixed infection with the GRD agents. The identification of GRSV in groundnut fields in Ghana highlights the need for its inclusion in future groundnut-breeding programs. Genetic diversity analysis of the coat protein gene of GRAV revealed more than 97% nucleotide and amino acid identity among the Ghanaian, Nigerian and Malawian isolates, providing a good opportunity for the development of pathogen-derived resistance to combat the disease.

#### **Detection, Distribution and Effect of Hop stunt viroid on different hop cultivars**

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Hop stunt disease caused by Hop stunt viroid (HSVd) is a growing threat to hop cultivation globally. It severely impacts growth and yield of certain hop cultivars. In 2004, HSVd was first detected in hop yards of Washington State, the major hop growing area in the United States. RT-PCR has been the major technique used for HSVd diagnosis; however, it is costly and sample handling is technically challenging. A more robust Reverse Transcription-Recombinant Polymerase Assay (RT-RPA) was developed to facilitate larger sample numbers. To understand the current distribution of HSVd in Washington, a survey was conducted and it revealed that 17% of hops grown in this region are infected with HSVd. Symptom expression is highly variable among different hop cultivars infected with HSVd. To understand the effect of HSVd, a 5 year study was conducted of six cultivars of hop to determine the impact on yield. Over the study period, the average cone yield of infected cultivars Glacier, Cascade and Willamette was reduced by 62%, 14% and 34%, respectively, compared to non-inoculated healthy plants. No significant yield reduction was observed in cultivars Nugget, Columbus and Galena cultivars. In the final year 2013, horticultural parameters of Willamette and Nugget were also measured.

#### **Discovery of various dsRNA mycoviruses in *Trichoderma* spp. causing green mold disease of shiitake *Lentinula edodes***

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A total of 315 fungal isolates causing green mold disease were collected from contaminated artificial logs and sawdust bags used for cultivating shiitake mushroom *Lentinula edodes* in Korea and were analyzed for the presence of dsRNA. dsRNA, which was purified using dsRNA-specific chromatography and verified by dsRNA-specific RNaseIII digestion, was detected in 32 isolates. The molecular taxonomy of dsRNA-infected isolates based on the ITS region of the rDNA indicated that all isolates belonged to the *Trichoderma* spp. The number and size of dsRNAs varied among isolates and the band patterns could be categorized into 15 groups. The most common dsRNA group, groupVI, occurred in 10 isolates encompassing three species of *Trichoderma*. Partial cDNA clones were obtained from two selected dsRNA groups and the sequence comparison of the cloned fragments of these dsRNAs revealed a high degree of similarity to sequences of a hypothetical protein and polyprotein genes of other mycoviruses, indicating the existence of mycoviruses in *Trichoderma* spp. Northern blot analysis using cloned cDNA showed specific hybridizing patterns in the dsRNA bands for isolates from which the clones were obtained, suggesting that many different mycoviruses, which have not been identified yet, exist in *Trichoderma* spp.

#### **Mixed Infection of Two Economically Important Tospoviruses in *Nicotiana benthamiana***

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*Tomato spotted wilt virus* (TSWV) and *Impatiens necrotic spot virus* (INSV) are two economically important tospoviruses. Mixed infection of TSWV and INSV was first reported on tomato plants from Italy in 2000; and mixed infection has also been found in tobacco in many states in the US for more than two years. Sialer and Gallitelli suggested that mixed infection in tomato could represent progressive adaptation of INSV to vegetable crops. To test this hypothesis, healthy *Nicotiana benthamiana* plants were mechanically inoculated with different ratios of TSWV/INSV and examined for how mixed

infections influence disease development. After two weeks, systemically infected leaves were collected to measure the viral titer by ELISA. Results show that TSWV is a poor competitor compared to INSV, since INSV could establish infection and reach high titer even when inoculated with equal amount of TSWV inoculum, but TSWV was not able to infect *N. benthamiana* efficiently in the presence of INSV. Mixed infections of tospoviruses are commonly found in nature, but the interaction between these two economically important tospoviruses and how mixed infections affect viral host range have not been studied yet. Our results provide insights into the interaction between TSWV and INSV in *N. benthamiana* and basic knowledge for future tospovirus mixed infection studies.

#### **Complete genome sequence of a *Watermelon mosaic virus* isolate from watermelon after 50 years of its detection in the United States of America**

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*Watermelon mosaic virus* (WMV) belong to one of the important plant virus genus *Potyvirus*. This virus has been detected for the first time in 1965 in the Rio Grande Valley of Texas but so far no complete genome of WMV is available from cucurbits in the United States (US). WMV isolate (TX29) was collected from Rio Grande Valley of Texas during a survey in 2010 which showed severe mosaic in a watermelon plant. The complete genome of WMV-TX29 isolate was found to be 10,049 nucleotides excluding the poly-A tail. Nucleotide sequence comparison of TX29 isolate showed more sequence similarities with a WMV-French isolate. This is the first report of the complete genomic sequence of a WMV isolated from cucurbits in the US.

#### **Interactions between *Bell pepper endornavirus* and acute viruses**

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Persistent plant viruses do not cause detectable symptoms in their plant hosts. In contrast, acute viruses cause symptoms, and in most cases, disease. Most bell pepper (*Capsicum annuum*) cultivars are infected with the persistent virus *Bell pepper endornavirus* (BPEV). To study possible interactions between endornaviruses and acute viruses, we developed two near-isogenic lines of the bell pepper cultivar Marengo; one BPEV-infected and the other BPEV-free. The two lines were mechanically inoculated individually, with a mild (M) and a severe (S) isolate of the acute virus *Pepper mild mottle virus* (PMMoV). Symptoms were recorded and relative amounts of PMMoV determined by ELISA. When the two lines were inoculated with PMMoV-M, all plants reacted with mosaic symptoms. In contrast, when PMMoV-S was inoculated, BPEV-free plants reacted with systemic necrosis and mosaic while BPEV-infected plants reacted only with mosaic. Results of the ELISA test showed that plants of the BPEV-infected line yielded consistently less PMMoV than plants of the BPEV-free line; however, the differences were not statistically significant. The two bell pepper lines were also inoculated with *Tomato spotted wilt virus*, *Potato virus Y*, *Cucumber mosaic virus*, and *Tobacco mosaic virus*, and in all cases, plants reacted with similar symptoms. Results obtained in this investigation suggest that BPEV inhibit the systemic necrotic reaction of Marengo bell pepper caused by PMMoV-S.

#### **Characterization of recombinant *Potato virus Y*<sup>NTN</sup> (PVY<sup>NTN</sup>) isolates from Sulawesi, Indonesia**

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In the last three decades, Granola, a potato cultivar originally from Germany, has become the most common cultivar for fresh consumption in Indonesia. In August 2014, a survey was conducted in Sulawesi, where potato fields cultivated with Granola and its selection, cultivar Super John were surveyed for *Potato virus Y* (PVY) presence using visual symptoms and serology. PVY was found in Sulawesi for the first time. Samples determined to be positive for PVY were subsequently typed to strain using RT-PCR assays. All PVY isolates sampled were identified as PVY<sup>NTN</sup> recombinants, with three recombination junctions in P3, VPg, and CP regions of the genome. Three local PVY isolates were subjected to whole genome sequencing and subsequent sequence analysis. The whole genomes of the Indonesian PVY<sup>NTN</sup> isolates I-6, I-16 and I-17, were found to be closely related to the European PVY<sup>NTNa</sup>. This recombinant type was shown previously to cause potato tuber necrotic ringspot disease (PTNRD) in susceptible potato cultivars. The dependence of potato farmers on mostly a single cultivar, Granola, may have given a competitive advantage to PVY<sup>NTN</sup> over other PVY strains resulting in the predominance of the PVY<sup>NTN</sup> recombinant. The dominance of PVY<sup>NTN</sup> in Sulawesi, and possibly in Indonesia as a whole, represents a potential risk to any newly introduced potato cultivar to the country, especially cultivars susceptible to PTNRD.

#### **Variation in aphid abundance and *Potato Virus Y* incidence in Oregon potato**

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*Potato virus Y* (PVY) is an economically important potato pathogen that can induce potato tuber necrotic ringspot disease. More than 25 aphids species are reported to vector PVY in a non-persistent manner. Green peach aphid (GPA, *Myzus persicae*) and bird cherry-oat aphid (*Rhopalosiphum padi* L.) are considered to be the primary PVY vectors. In 2015, aphid abundance and PVY incidence were measured in nine commercial potato fields in Oregon. Aphids were collected weekly, counted and identified based on morphological characteristics. Potato leaf tissue was sampled from each location biweekly and assayed for PVY using ELISA. PVY strain composition of ELISA positive samples was determined using an RT-PCR-based strain-typing methodology. Potato aphid (*Macrosiphum euphorbiae*) and GPA were less abundant than all the other unidentified aphids combined. PVY incidence was highest in Klamath (8.4%), followed by Umatilla (3.3%), Union (1.75%), and Morrow (0%) counties. Strain typing of the ELISA positive samples revealed the PVY<sup>NO</sup> strain to be most prevalent (56%) followed by PVY<sup>O</sup> (10.34%), PVY<sup>NTN</sup> (5.74%) and a mixed infection of PVY<sup>NTN</sup> and PVY<sup>NO</sup> (1.14%). Potato fields with border crops as sudan grass had lower PVY incidence and fewer aphids captured when compared to fields without border crops. Also, traps placed in fields adjacent to hay crops captured more aphids and had higher PVY incidence when compared to traps placed in fields adjacent to maize, mint, sunflower or wheat.

#### **Isolation and characterization of FRs551, a bacteriophage associated with the phyto bacterium *Ralstonia solanacearum* strain UW551**

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*Ralstonia solanacearum* species complex members are soil-borne, plant pathogenic bacteria that cause devastating diseases in a wide range of economically important crops around the world, with global losses in potato alone exceeding \$950 million annually. The race 3 biovar 2 strains of *R. solanacearum* causing potato brown rot are listed as select agent pathogens due to their potential threat to U.S. agriculture. Genome analysis of the select agent strain UW551 revealed the presence of a putative intact prophage sequence with high homology to the family *Inoviridae*. Active virions of this phage, designated ΦRs551, were isolated from the supernatant of UW551 cells grown under normal culture conditions and were found to infect other *R. solanacearum* strains. Electron microscopy showed that ΦRs551 is a filamentous-type phage approximately 1200 nm long and 7 nm wide. ΦRs551 has a circular DNA genome that is 7929 nt in length, with 100% identity to the putative prophage sequence in the UW551 genome. It is most closely related to ΦRS603, another filamentous phage of *Ralstonia* isolated from soil. Our draft ΦRs551 genome contains open reading frames (ORFs) similar to those found in other *Inoviridae* members, but has a unique overall ORF makeup not found in any other phage relative.

### Identification of conserved sequences within tospovirus genomes for broad-spectrum resistance using RNA interference (RNAi)

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*Tomato spotted wilt virus* (TSWV) and other tospoviruses cause significant yield losses in numerous crop species worldwide. Genetic resistance is the most effective management tool for tospovirus diseases in tomato; however, resistance breaking isolates of TSWV have emerged which are capable of overcoming the currently deployed resistance genes. An alternative to traditional resistance genes is transgenic resistance through the process of RNA interference (RNAi). Though effective against tospoviruses, the sequence-specific nature of RNAi means that resistance can break down if host plants are challenged with genetically diverse populations of tospoviruses. To examine viral sequence diversity, we used publicly available viral sequences of TSWV and other tospoviruses. Our approach focused on identifying short, highly conserved regions among 2679 TSWV sequences and across 589 additional sequences from seven tospovirus species. This analysis allowed us to locate sequence conservation within each of the five tospovirus genes. The identified sequences corresponded to motifs of known biological importance. Based upon their high conservation at the nucleotide level, correspondence with known biological functions, and low potential for off-target effects in tomato, these sequences have been incorporated into anti-viral RNAi constructs for tomato transformation.

### Correlation of endogenous pararetrovirus with symptoms of citrus blight disease

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Citrus blight (CB) is a major economic problem in citrus groves in Florida. Even though the disease was first described in Florida in 1894 the causal agent of CB is still unknown. A number of biotic and abiotic agents have been postulated as potential causes of CB. CB occurs in a wide variety of locations with diverse environments and is root graft transmissible, suggesting that the disease is caused by a systemic infectious biotic agent. Recently, we documented a population of endogenous pararetrovirus (EPRV) sequences in a root stock susceptible to CB, carrizo citrange. Five EPRV sequences were obtained from healthy, huanglongbing (HLB)-infected and blight-affected carrizo citrange, but only one pararetrovirus sequence, termed Citrus blight associated pararetrovirus (CBaPRV), was found as RNA in all CB affected plants but not in any of the HLB diseased or healthy plants. To assess the correlation between active CBaPRV RNA and CB, samples from 8 healthy trees and 42 citrus trees diagnosed as blighted using the syringe injection technique were collected from four locations in Florida. Reverse transcription-PCR with primers specific to CBaPRV amplified the active RNA from CB affected roots, leaves, bark and flowers, but not from any healthy or HLB infected tissues. While there is no direct evidence that CBaPRV is the causal agent of blight, the presence of viral RNA is tightly correlated with CB symptoms.

### Genetic diversity, host range and disease resistance to the emerging Tomato mottle mosaic virus on tomato

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Since its first discovery in 2013 in Mexico, Tomato mottle mosaic virus (ToMMV), a new tomato-infecting tobamovirus is now present in a number of countries (i.e., Brazil, China, and Israel) and several states in the U.S. There is little information available on the molecular and biological properties of this emerging virus. With approximately 80-85% nucleotide sequence identity to two other common tobamoviruses, Tomato mosaic virus (ToMV) and Tobacco mosaic virus (TMV), there is a need to understand the genetic diversity and serological and biological properties of this emerging virus in order to develop a species-specific detection method, and to assess disease resistance durability in tomato cultivars. Comparative host range evaluation showed similarity in host species infected by three tobamoviruses. However, ToMMV was more aggressive on host plants than that of TMV and ToMV. To evaluate whether the emerging ToMMV could break the disease resistance, three commercial tomato cultivars were screened along with control Moneymaker tomato through mechanical inoculation. We observed different responses in tomato cultivars to the three tomato-infecting tobamoviruses. ToMMV exhibited aggressiveness and broke the resistance in a tomato cultivar that had demonstrated full resistance to both TMV and ToMV. This resistance breaking in tomato by the emerging ToMMV presents a serious concern for tomato growers. The mechanism of such resistance breaking will be discussed.

### Diversity of aflatoxin-producing fungi in non-agriculture soils of Zambia

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Aflatoxins are cancer-causing, immunosuppressive toxins that contaminate maize and groundnut, important staples in Zambia. *Aspergillus flavus*, *A. parasiticus* and two unnamed taxa are frequently implicated as causal agents of contamination in Africa. Most of Zambia's arable land is uncultivated. *Aspergillus* communities in crops may have originated in uncultivated regions and soils in these areas may serve as reservoirs of toxin-producers. Understanding fungal communities in cultivated and uncultivated areas may facilitate aflatoxin management. Soils from fields cropped to maize and groundnut (161) and from uncultivated land (53) were assayed for *Aspergillus* section *Flavi* from 2012 to 2016. Communities in both wild and cultivated soils were dominated by *A. parasiticus* (90% and 50%, respectively) and the *A. flavus* L morphotype (5% and 31%, respectively). Phylogenetic analyses with partial gene sequences for nitrate reductase (*niaD*, 1.8 kb) and the aflatoxin pathway transcription factor (*aflR*, 2.1 kb) resolved 20 *A. parasiticus* isolates from cultivated soils into 4 well supported lineages. Relationships among the 4 lineages and *A. parasiticus* from other regions were evaluated. Impacts of fungi present prior to introduction of crops may cause differences in the etiology of aflatoxin contamination among regions. Characterization of fungi from non-cultivated soils may provide insight on which fungi are best adapted to the region and allow for improved aflatoxin management.

### Differential effects of DON and 15-ADON on maize seedling growth

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*Fusarium graminearum* (Fg) is a common pathogen causing maize seedling blight in China. Trichothecene mycotoxins produced by Fg have been implicated as pathogenicity factors in seedling blight, but little is known about the effects of specific toxins, such as DON and 15-ADON. In order to study the effect of both mycotoxins on seed germination, maize seeds were soaked in 20 µg ml<sup>-1</sup> of DON or 15-ADON for 12h and germinated by rolled paper method; seed germination was counted daily and root length and seedling weight were measured after 7 days. The results showed both mycotoxins decreased seed germination significantly compared to the control, but there was no significant difference between DON and 15A-DON treatments. However, 15 A-DON displayed significantly reduced root length and lateral root numbers compared to DON. We speculated that 15-ADON production by *F. graminearum* in early stages of infection plays an important role in the poor development of roots, which is a typical symptom of maize seedling blight in the field.

### Diversity of aflatoxin producing fungi in Malawi

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Aflatoxins are toxic fungal metabolites that often contaminate Malawi's food and feed. These toxins cause immune suppression, stunting, and cancer. *Aspergillus flavus* and *A. parasiticus* are the most frequently reported causes of contamination. Aflatoxin producers vary widely in genetic diversity, aflatoxin production, and agro-ecological preference. Little is known about the diversity of fungi causing aflatoxin contamination in Malawi. The current study examined DNA variability among aflatoxin producers in Malawi. Maize and groundnuts were sampled across the five agro-ecological zones of Malawi and fungi infecting crops and belonging to *Aspergillus* section *Flavi* were identified. The *A. flavus* L morphotype comprised 44% of the infecting fungi. Fungi with S morphology composed 40% and *A. parasiticus* made up 15%. Use of molecular phylogenetics to assign S morphology fungi to species is underway. Fungi that did not produce aflatoxins (793) and belonged to the *A. flavus* L morphotype were characterized at 17 microsatellite loci in order to identify fungi of potential value as biocontrol agents for the aflatoxin prevention. 350 microsatellite haplotypes were detected. Knowledge of variability among the aflatoxin producers may also provide opportunity to improve aflatoxin management.

#### **Effects of nutrients and microorganisms on aflatoxin production by *Aspergillus flavus* during successive generations of subculturing**

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*Aspergillus flavus* infects major agricultural crops, and produces aflatoxin which has been linked to increased liver cancer in humans. Factors such as nutrition, pH and temperature are known to influence *A. flavus* growth and subsequent aflatoxin production. However, the long-term effects of various media and the presence of other microorganisms on aflatoxin production have not been well studied. In the present study, *A. flavus* AF13 strain was grown on media containing different levels of nutrients, such as V8, Potato Dextrose Agar (PDA) and Luria Broth (LB), and subcultured for 56 generations. The highest aflatoxin production was observed with *A. flavus* grown on PDA, which was about 100 times higher than on LB or V8. Aflatoxin production appears to increase with successive transfers of *A. flavus* on the PDA medium. On the other hand, aflatoxin production seems to decrease slowly with subsequent transfer when *A. flavus* is grown on V8 or LB medium. At the 39th generation on LB medium, aflatoxin production decreased to a level that was undetectable with HPLC. *A. flavus* that produced no detectable aflatoxins on LB medium regained the ability to produce high levels of aflatoxin when transferred from LB to PDA medium. These results confirmed earlier studies which have demonstrated that nutrients, especially the carbohydrates in PDA, play a key role in aflatoxin production over time. The impact of microorganisms on aflatoxin production in LB and V8 media will be discussed.

#### **Mycotoxins contamination on hybrids and landraces of stored maize grain in México**

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Mycotoxins contamination is a human and animal health problem because the exposure to such substances present on grains infected by several fungi can cause diseases; thus this work explored mycotoxins contamination levels on maize grain in México. A survey was conducted in six locations forming three sets of samples including hybrids and landraces collected by UAAAN, a seed enterprise and cooperating farmers. 31 samples were tested for moisture content (MC) by constant temperature oven method; phytopathogenic fungi detected and counted by freeze blotter test, and aflatoxins B1, total fumonisins and deoxynivalenol contamination levels determined with inverted ELISA test (Enzyme-Linked-Immune-Sorbent-Assay) (Romer Labs®). General mean of MC was 11.63%. Potentially toxigenic phytopathogenic fungi detected were *Fusarium verticillioides* 7.6%, *Fusarium graminearum* 2%, *Aspergillus* spp. 9.2% and *Penicillium* spp. 1.1%. Mycotoxins levels were 0.106-2.665 ppm of total fumonisins, 0.64-2.67 ppm of deoxynivalenol and 0 ppb of aflatoxins B1. Mycotoxins levels detected on maize grain were safe for consumption according to Food and Drug Administration (FDA) recommendations, which are: 2-4 ppm of total fumonisins, 5-10 ppm of deoxynivalenol and 20 ppb of aflatoxins B1. Some mycotoxins detection levels were near the FDA risk values, indicating the need to regulate also other mycotoxins than total aflatoxins, which currently is the only one regulated by the Government of México.

#### **Profile of *Aspergilli* in fields under continuous maize and groundnut cultivation and aflatoxin contamination along the crop value chains in Mozambique**

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Aflatoxins are routinely reported in maize and groundnut in Mozambique, but the profile of aflatoxigenic fungal isolates in the fields continuously cultivated with maize or groundnut and the extent of aflatoxin accumulation along the maize and groundnut value chains are unknown. Georeferenced soil samples were taken from 250 farmers' fields at harvest in 2013 and 2014 in different agro-ecological zones (AEZ) to determine *Aspergilli* profile. The fields had been under continuous maize or groundnut cultivation from two to five years. Also, aflatoxin content was measured in 300 maize and groundnut samples collected along the crops' value chains. Inoculum density of *Aspergilli* was high (>2,000 CFU/g) in fields under extended (≥3 years) continuous cultivation, and low-medium (0-2,000 CFU/g) either in fields with <3 years of consecutive cultivation, or at high altitude AEZ (>1,000m), irrespective of crop. *Aspergillus flavus* S (34%) and L (31%) morphotypes were most frequently isolated from fields under intensive cultivation (≥3 years), but L morphotype was predominant (>50%) in fields with <3 years of continuous cultivation, irrespective of crop or AEZ. Aflatoxin content was high in groundnut leftovers used for human consumption after sorting (max. 3,874 ppb), groundnut from storage (max. 1,777 ppb) and groundnut sold at marketplaces (max. 720 ppb). These results suggest that targeted aflatoxin management practices may be required along the crop value chains in Mozambique.

#### **Influence of corn GMO traits for insect control on aflatoxin and fumonisin contamination in Texas**

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Contamination of corn with aflatoxin and fumonisin is a chronic problem throughout most of Texas. Insect damage to ears can be a predisposition factor, so controlling insects using GMO traits could be a management tool. The objective of our field experiments was to evaluate the impact of three different GMO traits in one parent hybrid (N78S) on mycotoxin contamination. Hybrids were planted in two-row, 22 ft-long plots in a randomized, complete block design near College Station in 2014 and 2015 and were inoculated with either *Aspergillus flavus* or *Fusarium verticillioides* or both. Overall, in 2014, the Viptera-trait hybrid expressing *Cry1Ab* and *Vip3Aa20* Bt proteins accumulated less aflatoxin (28 parts per billion (ppb) average, range 1-84 ppb) than hybrids that expressed only *Cry1Ab* (161 ppb, range 38-340 ppb), or no Bt proteins (175 ppb, range 54-410 ppb). Fumonisin was significantly ( $P=0.05$ ) reduced in the Viptera-trait hybrid (0.3 parts per million, ppm) compared with the other hybrids (5.5 ppm and 7.5 ppm). In 2015, there was greater drought stress at flowering, which increased overall aflatoxin levels. The Viptera hybrid had the lowest average aflatoxin, 147 ppb, compared with the other hybrids (346 ppb and 274 ppb). The average fumonisin was 3 ppm with the Viptera hybrid, compared with the other hybrids (9.5 ppm and 12 ppm). These results show that in some years, the insect-control traits in some GMO hybrids will not be sufficient to reduce aflatoxin.

### **Conidial versus sclerotial production: Implications for corn and soil niche specialization in *Aspergillus flavus***

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*Aspergillus flavus* contaminates corn and other oil-seed crops with carcinogenic aflatoxin. The populations of *A. flavus* differ between soils and crops. In Louisiana, native *A. flavus* isolated from soil and corn in 11 fields revealed only 6 of 16 vegetative compatibility groups (VCG) infected corn. During inoculum production for an infectivity titration experiment to verify niche specialization, two predominant VCGs in corn produced approximately 15 times more conidia than the most widespread soil VCG. Instead of producing conidia, the widespread soil VCG produced copious sclerotia. To test the hypotheses that corn infecting strains produce more conidia and soil inhabiting strains more sclerotia, three isolates from each of 16 VCGs were grown on Czapek Dox and Wickerham solid media and assessed for conidial or sclerotial production respectively. Generally corn VCGs produced more conidia; two VCGs comprising 91% of corn isolates produced significantly more conidia (3-60X) than 9 of 10 strictly soil VCGs. Conversely, those two corn VCGs produced very limited sclerotia, whereas all strictly soil VCGs produced significantly more sclerotia (10-100X). High conidial production appears to correlate with corn infection, whereas production of resting bodies appears to correlate with survival in the soil. Suppression of conidial production toward sclerotial production may reduce corn infection and limit outbreaks of aflatoxin contamination in corn.

### **Evaluation of sorghum germplasm for resistance to grain mold and mycotoxin contamination in the Mid-Atlantic**

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Grain sorghum production is increasing in the Mid-Atlantic region due to demand for locally sourced animal feed. Grain mold, caused by a complex of fungal species, infects grains, deteriorates quality, and reduces yield 30-100%. Some grain mold fungi produce mycotoxins that are toxic to both humans and animals, but little is known about the fungi and mycotoxins associated with sorghum grain in the Mid-Atlantic. The objective of this study was to evaluate sorghum germplasm for susceptibility to grain mold and mycotoxin contamination. Under natural field conditions, 375 genetically diverse sorghum accessions from more than 25 countries were evaluated for agronomic traits (panicle type and grain color), grain mold severity, and deoxynivalenol (DON) contamination. Mold severity ranged from 0-90%, and DON ranged from undetectable to 10 ppm. Grain mold severity and DON concentrations varied among panicle types and grain colors ( $P < 0.001$ ). Overall, open panicles with dark colored grains had lower mold severity and DON concentrations than compact panicles with light colored grains. Given the potential for grain mold and DON contamination in sorghum grown in the Mid-Atlantic, future work will focus on characterization of the causal agents of grain mold and their associated mycotoxins and identification of sorghum germplasm and agronomic traits associated with reduced susceptibility to mold and mycotoxin contamination.

### **Seasonal population dynamics of mycotoxigenic *Aspergillus* section *Nigri* species on grapes and in vineyard soil using ddPCR**

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Several species of *Aspergillus* section *Nigri* (black Aspergilli), including mycotoxin producers, are common residents of grape vineyards, but measuring relative population sizes of individual species using traditional isolation and identification methods is impractical. We developed species-specific primers and probes for quantitative droplet digital PCR (ddPCR) to simultaneously detect and quantify *A. niger*, *A. carbonarius*, *A. welwitschiae* and *A. tubingensis* populations from grape and soil samples. Samples were collected at berry set, veraison, harvest and raisin stages from two vineyards over two consecutive years. Plate counts showed that total fungal counts were  $10^2$ - $10^5$  cfu/g on berries and  $10^1$ - $10^5$  cfu/g in soil. Plate counts of black Aspergilli in soil were between  $10^2$  and  $10^4$  cfu/g, independent of sampling time, while counts of black Aspergilli on berries increased from  $<10^1$  cfu/g at berry set to  $>10^3$  cfu/g on raisins. Distribution of *Aspergillus* species using ddPCR showed that in soil, relative abundance of each species varied widely among samples, but *A. niger* was the most abundant species in 52 of 60 samples. On grapes, relative abundance of *A. carbonarius* was higher on mature grapes and raisins, and correlated with ochratoxin levels in those samples. This ddPCR method is a useful tool to describe fungal population dynamics in the environment and to monitor mycotoxigenic *Aspergillus* species during grape and raisin production.

### **Factors influencing *Aspergillus flavus* community structures in fields treated with the atoxigenic biocontrol *A. flavus* AF36**

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Aflatoxins, toxic and carcinogenic metabolites produced by several fungi in *Aspergillus* section *Flavi*, are perennial contaminants of cottonseed in Arizona and impact crop value. Atoxigenic strains of *A. flavus* have been used to displace aflatoxin producers and manage aflatoxins in cottonseed in Arizona for over two decades. Previous work indicated applications benefit both treated and subsequent crops and that atoxigenic strains in treated soils decrease over time to reach equilibrium with the native *A. flavus* community. Crop rotation has been implicated as a factor influencing atoxigenic strain retention. The present study sought to determine factors influencing both retention of the biocontrol AF36 and reestablishment of the S strain. Weather, soil type and geography were all found to influence the community structure of *A. flavus* with hot temperatures, heavy soils, and low elevations favoring the S strain. Regression analyses indicated significant differences among regions in rate of change of the populations of both AF36 and the S strain as a function of time after application. Populations of *A. flavus* in soils are highest immediately after harvest. Steep declines occur during the few months following harvest. Both the S strain and AF36 followed similar patterns, fitting an exponential decay model. However, quantities of S strain propagules decline slower than AF36, suggesting the S strain may be adapted to resist soil factors that reduce *A. flavus* viability.

### **Correlation between the disease incidence of Fusarium head blight and the mycotoxin content of winter cereal crops in Korea**

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In 2015, diseased grain rates of Fusarium Head Blight of winter cereal crops in southern region of Korea were 9.5% at an average and the incidence is slightly higher than compared to the previous year's. Diseased grain rates of Fusarium Head Blight on barley were 18.8% in Jeollanamdo province and that was the highest in Boseonggun with 26.4%. Diseased grain rates of Fusarium Head Blight on wheat was 19.2% in Jeollanamdo province and 12.9% in Jeollabukdo province. It showed the higher incidence in Haenamgun, Goseonggun especially with 35%. Mycotoxin content was investigated from the seed samples which have been collected by each region. Deoxynivalenol was 13.3 mg/kg in Sacheon city and 16.3 mg/kg in Goseonggun which was the higher disease incidence than the other regions. Zearalenone contents of the seed samples which have been collected in Sacheon city, Buangun, and Gangjingun as the highest incidence region were 1.15 mg/kg, 1.11 mg/kg, 1.62 mg/kg respectively. Fumonisin contents of the seed samples which have been collected in Sacheon city, Boseonggun, and Haenamgun were 2.54 mg/kg, 2.19 mg/kg, 2.64 mg/kg respectively. Correlation coefficient between the diseased grain rate of Fusarium Head Blight and the Zearalenone content was 0.81.

### **Analysis of deoxynivalenol and deoxynivalenol-3-glucoside in Canadian spring wheat varieties inoculated with *Fusarium graminearum***

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Mycotoxins are a chemically diverse group of toxic secondary metabolites produced by fungi. In order to cope with these mycotoxins, plants have the potential to modify the chemical structures as part of their defense mechanism. The resulting metabolic products are neither routinely screened at grain



elevators nor regulated by proper legislations. The objective of this study is to analyse the deoxynivalenol (DON) and deoxynivalenol-3-glucoside (D3G) content in Canadian spring wheat varieties grown in two locations, inoculated with different chemotypes of *Fusarium graminearum*. According to the analysis of variance, significant differences were observed among the varieties for fusarium head blight (FHB) disease index, fusarium damaged kernel percentage (%FDK), DON and D3G content. When considered the effect of chemotype, significant differences were observed for FHB disease index, %FDK and DON content. The D3G content and D3G/DON ratio were not significantly different between the chemotypes. Correlation analysis showed a strong positive correlation between DON and D3G. The highest D3G/DON ratio was observed in moderately resistant varieties. The susceptible varieties showed lower D3G/DON ratio compared to the moderately resistant varieties. The current study indicated that Canadian spring varieties produce D3G upon *Fusarium* infection and testing for D3G in food and feed safety assessments in Canada should be considered.

#### **Seed Quality of Maize Cultivars Infected With *Pantoea agglomerans***

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*Pantoea agglomerans* is an important seed borne pathogen of maize. There are reports about transmission rates, however, information concerning the quality seed is weak. The aim of this study was to evaluate the quality of seed produced in infected plants with *Pantoea agglomerans*. Dent corn hybrids Triunfo (comercial) and 9Bx52 (experimental) were sown in Experimental Station of Colegio de Postgraduados, Montecillo, Mexico. Disease assessments were made once a week during crops cycle. Seeds were harvested and separated according severity of mother plant (healthy, non-systemic and systemic). Seed quality was evaluated with standard test germination in laboratory and vigour in greenhouse. Disease incidences of systemic infection were 3.6% for Triunfo and 2.8% for 9Bx52. The results show that 1000-seed weight was lower in systemically infected plants. All variables in laboratory and greenhouse were significantly lower in seed plants with systemic infection. These results indicate that the severity of infection from the mother plant affects the quality of the seed.

#### **Rapid detection of preexisting internal *Leuconostoc* spp. spoilage populations in fresh-cut carrots during storage**

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During an extended period of abnormally short quality retention in mixed component packaged salads, due primarily to rapid decay, a root-cause investigation was undertaken. From an initial investigative assessment, this study focused on identification of the primary underlying microbiological cause and suggested the need for a rapid detection screening of raw material. Analysis of several lots of unprocessed product, approx. 6 cm abrasively peeled carrot plugs, revealed the accumulation of an aqueous slime in the shipping bag void space and around the extremely softened plug surfaces. This premature diagnostic sign of lactic acid bacteria (LAB) spoilage, specifically *Leuconostoc* spp., developed in cold storage (2.5°C) after two weeks. Efforts were undertaken to determine whether the *Leuconostoc* was internalized in raw material or primarily environmental contamination with a proliferating reservoir of LAB in the primary processing and packaging environment. Polymerase chain reaction (PCR) primers specific for the amplification of a sequenced region of the *Leuconostoc* 16S ribosomal RNA gene confirmed the taxonomic identity. Total initial LAB and *Leuconostoc* bacterial populations isolated from symptomatic carrots ranged from log<sub>10</sub> 7.5–8.5 and log<sub>10</sub> 3.5–4.0 CFU/g carrot tissue weight respectively, and increased log<sub>10</sub> 2 CFU/g log<sub>10</sub> and 3.5 CFU/g respectively in population density on asymptomatic and symptomatic raw carrot material during two week refrigerated storage.

#### **Controlling bacterial soft rot in tomato fruit that have been inoculated through lenticels located around the stem attachment**

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Tomato fruit harvested from wet plants or exposed to rainfall prior to packing have a high potential for postharvest decays. In laboratory tests, bacterial soft rot (*Pectobacterium carotovorum*) development during storage of fruit inoculated in the stem depression was delayed by air-drying, wiping dry, or chlorine washes with efficacy linked with the interval between inoculation and treatment as well as type of treatment. Fruit that were exposed to water alone did not develop decay during storage. Incidence of bacterial soft rot was rare (0 to 13%) among inoculated fruit that were washed with chlorinated water, or wiped dry within 10 sec after dip inoculation, whereas lesions developed among > 50% of fruit washed or wiped dry at 60 min post inoculation. Incidence among non-treated controls was 100% within the 5-day storage. In three separate experiments, bacterial soft rot was reduced by an average of 52, 60, and 20% when fruit were air-dried (30 min) at 1, 4 or 18 h after inoculation, respectively. Corresponding values for washing in chlorinated water (200 ppm, pH 6.5) for 1 min averaged 48, 40 and 2%, respectively. Although evidence does not indicate that the pathogen is introduced into stem scar infection courts by contact such as occurs with wound inoculation, interventions must be initiated within an hour or less after stem scar exposure to inoculum in order to minimize the decay potential.

#### **Assessment of hermetic storage of maize under different environmental conditions**

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Storage of maize is a significant challenge for farmers in developing countries. Fungal growth and mycotoxin accumulation are some of the leading causes of postharvest losses, which are estimated to be \$4 billion annually in the Sub-Saharan. One of the largest postharvest threats is the growth of *Aspergillus flavus* and the accumulation of aflatoxin. As maize production in Africa continues to grow, so does the need for storage methods that will help mitigate postharvest losses. This study investigated the use of Purdue Improved Crop Storage (PICS) bags, a three-layer hermetic-storage system, for the prevention of grain rewetting in humid environments in order to mitigate fungal growth and aflatoxin accumulation. Maize (14% grain moisture) was stored in PICS and woven polypropylene bags in Indiana and Arkansas, two environments contrasting in temperature and relative humidity. After 3 months of summer storage, moisture in the woven bags increased significantly in both environments (as high as 15.9%). Populations of storage insects and fungi also increased in the woven bags, with the greatest change under the Arkansas environment. In contrast, grain in the PICS bags was not affected by the two environments; grain moisture remained unchanged, there were no storage insects, and fungal populations increased only slightly. These results provide evidence that PICS bags can be used for the safe storage of maize in the highly-variable environment of Sub-Saharan Africa.

#### **Bitter rot on apple: Comparative epidemiology of *Colletotrichum* species and growth temperature**

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The *Colletotrichum* genus was reclassified, and based on this new classification were described five species causing Bitter Rot on apple belonging to two complex, *C. acutatum* complex (CaC: *C. nymphaeae*, *C. paranaense*, *C. melonis*) and *C. gloeosporioides* complex (CgC: *C. fructicola*, *C. siamense*). However, are unknown the optimum conditions for the development and the impact of each species in the disease progress. Therefore, the aim of this study was to determine, for each species, the optimum temperature and evaluate the infection and colonization in fruits of the cultivar Gala. The optimum temperature for the development of the colonies was 22-24° C for CaC, and 25-26° C for CgC. The progress of the disease was significantly higher in fruit inoculated with CaC than in fruit inoculated with CgC. The incubation period was 2 to 3 days for all isolates in wounded fruit, while in unwounded

the period was 18-25 days. The latency period was 7 days to *C. paranaense* and 6 days to *C. nymphae* with fruit wounded but in unwounded fruit, only *C. paranaense* sporulates with higher latency period (33 days).

#### **Use of induced resistance for the management of postharvest decay of fruit**

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Up to 25-30% of fruit harvested nowadays is lost, and decay causing fungi are among the main causes of such losses. Harvested produce has high economic impact, covering the costs of production, harvesting, transport, eventual cold storage, exposure on the shelf of stores (so called shelf life) and final life at the home of consumer. During this period, the fruit can be exposed to a list of fungi able to take profit of storage conditions, with high humidity, low temperatures, and senescence, that make weaker the fruit reaction to the infection. The main postharvest pathogen is *Botrytis cinerea*, but considerable losses of fruit are caused, according to the species, by *Penicillia*, *Colletotrichum* spp., *Monilinia*, *Rhizopus* spp., *Mucor* spp., *Alternaria* spp., *Cladosporium* spp. and some other decay causing fungi. Nowadays the most frequent way to prevent postharvest decay relies in the use of synthetic fungicides, but considering their limitations, alternatives based on the use of biocontrol agents, physical means, decontaminating agents, and natural compounds are deeply studied and often used in the field and/or in packinghouses. Most of these alternative treatments have direct antimicrobial activity and at the same time they elicit the host defenses. The different ways to apply the induced resistance, with negative and positive aspects, mechanisms involved and tools useful to track the physiological changes occurring in the host tissues will be discussed.

#### **Susceptibility of sweetpotato (*Ipomoea batatas*) cultivars to fungal and bacterial diseases**

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Sweetpotato [*Ipomoea batatas*], is the number one vegetable crop in the state of North Carolina. This crop is a high-nutrient content food and a great source of pro-vitamin A, vitamin C, calcium, and iron. Field and post-harvest diseases cause yield and economical losses during sweetpotato production. Rhizopus soft rot, caused by the ubiquitous *R. stolonifer*, Fusarium root rot, associated with *F. solani* and *F. oxysporum* f. sp. *batatas* infections, and Streptomyces soil rot, caused by *Streptomyces ipomoeae*, are among the most detrimental diseases of sweetpotato in North Carolina. Management of sweetpotato diseases through application of antimicrobial chemical compounds is limited since few products are labeled for use in sweetpotato and due to residue restrictions for export markets. Development of cultivars with genetic resistance is the most desirable and economical means of managing plant diseases. The present work was conducted to evaluate sweetpotato genotypes for resistance to economically relevant post-harvest diseases in sweetpotato production. For this purpose, replicated greenhouse and growth chamber experiments were performed to screen for resistance and susceptibility of 16 sweetpotato varieties to 8 microbial isolates. The results suggested that sweetpotato genotypes differed in resistance to the tested phytopathogenic isolates.

#### **Development of edible composite coatings with antifungal GRAS salts for reduction of postharvest gray mold of cherry tomato fruit**

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GRAS (generally regarded as safe) salts were preliminary selected in *in vitro* tests against *Botrytis cinerea*, the causal agent of tomato postharvest gray mold, and added at 2% wet basis (wb) to emulsion matrixes prepared with hydroxypropyl methylcellulose (HPMC) and glycerol (ratio 3:1 dry basis, db), beeswax (BW) and oleic acid (ratio 5:1 db), and tween 80 (1.5% wb). The final emulsion solid concentration was modified to 7-10% wb in order to obtain formulations with a viscosity range of 100-150 cp. Selected stable coatings were tested *in vivo* against gray mold on cherry tomatoes cv. Josefina artificially inoculated with *B. cinerea* 24 h before coating (curative activity) and incubated at 20°C for up to 15 days. Among these edible coatings, the most effective were those containing sodium propionate (SP), potassium carbonate (PC), ammonium phosphate (APh), and ammonium carbonate (AC), and they were further tested *in vivo* with fruit subjected to cold storage. Gray mold incidence and severity were determined on cherry tomatoes inoculated with *B. cinerea*, coated 24 h later, and stored at 5°C for 7 and 14 days followed by a shelf life period of 7 days at 20°C. The antifungal activity of the coatings was fungistatic rather than fungicidal. Although the HPMC-BW coatings formulated with SP were the most effective in reducing gray mold on cold-stored cherry tomatoes, coatings containing AC were selected due to their better performance for overall fruit quality preservation.

#### **Analysis of IAA and ABA in HLB diseased citrus trees**

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HLB, caused by *Candidatus Liberibacter asiaticus* (Las), is a disastrous disease resulting in defoliation, twig dieback, root decline and fruit drop. Among these symptoms, preharvest fruit drop directly poses a disastrous threat to the citrus industry by decreasing yield. At early developmental stage of fruit, dropping always occur at AZ-A zone which is between branch and peduncle. Here, different hormones level at peduncle area were dynamically monitored both in healthy and HLB infected trees during the fruit maturation to find if hormone imbalance in HLB infected peduncles caused preharvest fruit drop. Indole-3-acetic acid (IAA) level was lower in HLB infected trees at the early stage of fruit ripening compared to healthy trees. Level of abscisic acid (ABA), which promote fruit mature and abscission, was higher in Las infected trees. Expression analysis of hormone related genes also matched the hormone detection. We will also report our results in preventing preharvest fruit drop using different hormones.

#### **Seasonal variation in the antibacterial activity of latex-like resin from *Sciadopitys verticillata***

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Japanese umbrella pine, *Sciadopitys verticillata*, produces a latex-like resin with antibacterial activity. To determine seasonal antibacterial activity, resin was collected in summer and winter of 2015, stored at -20°C, autoclaved twice, and suspended in sterile deionized water. Resin was tested within two months of collection. Bacterial suspensions (100 µl) from nutrient broth (NB) overnight cultures were mixed with NB (100 µl), treated with resin suspension, and incubated overnight at 26°C with shaking. Treatments were: 0 (control), 25, 50, and 100 µl resin suspension diluted in water (total volume 100 µl). Colony-forming units were determined with dilution plating. Separate tests were conducted with winter and summer-collected resins, and bacterial species were evaluated individually. Data (number of treatment colonies relative to control colonies) were analyzed with mixed models ANOVA. Differences were significant at  $P=0.05$ . Exposure to resin collected in winter reduced numbers of colonies of *Bacillus cereus*, *Erwinia amylovora*, *Agrobacterium tumefaciens*, and *Escherichia coli*, and increased numbers of *Xanthomonas campestris*, *Pseudomonas fluorescens* (Pf), and *Pseudomonas syringae*. Exposure to resin collected in summer affected population growth of two bacterial species; colony counts of *E. amylovora* decreased and those of *P. fluorescens* increased. Future research will address the role of chemical composition and resin concentration on antibacterial activity.

#### **Bioactive effect of triterpene extracts in mix with *Pseudomonas* to control *Gaeumannomyces graminis* var. *tritici* on wheat plants**

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Extracts rich in saponins from quillay (*Quillaja saponaria* Mol.), an endemic Chilean native tree, and rhizobacteria of the genus *Pseudomonas*, which produce antimicrobial compounds such as 2,4-Diacetylphloroglucinol (2,4-DAPG), have been associated with reduction of the fungus *Gaeumannomyces graminis* var. *tritici* (Ggt), causal agent of take-all disease of wheat. However, reports about bioactivity between these bacteria and this natural triterpenoids are not available. Through *in vitro* and *in vivo* tests the interaction between both new sources of take-all disease control were assessed. Results showed that quillay extracts Vet SAP® (>90% of saponins) and QL1000® (~8% of saponins) controlled Ggt *in vitro* and had a differential effect according to their purity on populations of 2,4-DAPG-producing *P. protegens*, with Vet SAP® not affecting the bacterial growth at 800 ppm of quillay saponins. On wheat seedlings, Vet SAP® did not affect the bacterial populations of the three bacterial strains assessed, although, quillay saponins at concentration of 7,360 ppm reduced bacterial biofilm formation on the rhizosphere. Strains of *P. protegens* had a variable antagonistic activity on wheat seedling roots and kept their disease control ability when were combined with different concentrations of Vet SAP®. These results suggest that mixtures of the antagonistic bacteria and plant extract to control *G. graminis* var. *tritici* could be useful in developing a biopesticide.

#### Protection of wheat against *Zymoseptoria tritici* using sugar beet extract-based resistance inducers

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In the current context of sustainable agriculture, the use of resistance inducers (RIs) is a promising approach to control crop diseases. A total of 73 putative RIs, including sugar beet (SB) extract-based products, phytohormones, vitamins, and amino acid-based RIs, such as GABA and BABA, were assessed for their efficacy on wheat against *Zymoseptoria tritici*, a major pathogen on this crop. The experiments were performed in the greenhouse using third-leaf stage plants of the susceptible cv. Alixan treated with each putative RI using foliar application two days before inoculation with the pathogenic strain T02596. Disease symptom scoring three weeks post-inoculation revealed significant reductions of leaf chlorosis and necrosis as well as sporulation (pycnidial density) in plants treated with saccharine, salicylic acid, abscisic acid, GABA, BABA, vitamins C and BX, probenazole and 33 SB extract-based RIs, such as RI2474, which was the most efficient molecule (up to 71% disease reduction). Among the 73 tested molecules, 25 displayed an *in vitro* inhibiting effect on *Z. tritici* growth. The three most efficient SB extract-based RIs without biocide effect, including RI2474, were selected in order to improve their efficacy by optimizing the dose, the formulation and application conditions. In addition, cytological, biochemical and molecular approaches will be set up to identify the plant defense mechanisms involved in the protection conferred by the three selected RIs.

#### Switchgrass Extractives Inhibit Plant Pathogenic Fungi

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Switchgrass is a leading contender for biofuel production. Cellulosic ethanol yield is reduced by a phenolic-rich, nonstructural portion of switchgrass, called extractives. Switchgrass extractives hinder enzyme activity and microorganism growth utilized during the biofuel conversion process. Switchgrass would be a more cost-effective feedstock if the extractives portion was developed for use as a biopesticide. The aim of this study was to assess different concentrations of switchgrass extracts for inhibition of *Fusarium oxysporum*, *Fusarium graminearum*, *Alternaria alternata*, *Botrytis cinerea*, and *Bipolaris oryzae*. Crude ethanol extracts were evaluated for inhibition of fungal plant pathogens with disk diffusion and spore germination assays. Colony growth was measured in disk diffusion assays on water agar; treatments were disks treated with 60X and 75X concentrations of extracts, and a 'no extracts' control. Inhibition of mycelial growth was greatest in disk diffusion assays with *A. alternata* and *F. graminearum* at 75X, and *F. oxysporum* at 60X. Spore germination was tested by incubating spores in a water control, or in extracts concentrated at 10X and 15X for 24 h, followed by repeated sampling over a 4-hour period. Germ tube growth was observed microscopically. Spore germination of all fungi was significantly inhibited by both extracts concentrations at all time points. Results indicate that switchgrass extracts have biopesticide potential.

#### Quantitative assessment of the efficacy of ZnO nanoparticles against selected fruit fungal contaminants

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The use of traditional antifungals to control postharvest diseases has been reviewed or banned during the past years. Nanoparticles represent an alternative for postharvest disease management, either as coating agents or embedded into warehouses' filters. The efficacy of zinc oxide (ZnO) was assessed following the identification of the optimal growth temperature of *Penicillium expansum*, *Alternaria alternata*, *Botrytis cinerea* and *Rhizopus stolonifer*. Fungi were inoculated ( $10^5$ - $10^6$  spores/mL) onto SDA plates and incubated at temperatures from 5°C to 35°C under constant  $a_w$ . Mycelium diameters were measured and then fitted to a linear model to estimate the growth rate ( $\mu$ ) and the apparent lag time ( $\lambda$ ). The properties of ZnO (3 to 15 mM) were evaluated for the previously identified optimal growth temperature by mycelium diameter and optical density (OD) measurements recorded every 20 min for up to 6 days with a 96-well microtitre reader at 600 nm. *P. expansum* and *B. cinerea* could grow even at 5°C while *R. stolonifer* had the highest  $\mu = 0.383 \pm 0.002$  (cm/h) at 25°C and the shortest  $\lambda = 17.703 \pm 0.228$  (h) at 18°C. The antifungal tests showed *R. stolonifer* and *P. expansum* as the most sensitive to ZnO being completely inhibited even at 3 mM. *A. alternata* and *B. cinerea* were inhibited at 12-15 mM. These results were confirmed by the OD assay. ZnO nanoparticles thus appear as an effective antifungal and their efficacy can be assessed by quantitative mycological studies.

#### Recommendation of Clove oil to minimize *Penicillium* based postharvest losses in pomegranates (*Punica granatum* L)

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Plant essential oils are important alternate to synthetic fungicides for the management of postharvest diseases in fruits. In the present study, among the tested clove (*Syzygium aromaticum*), coriander (*Coriandrum sativum*) and oregano (*Oreganum vulgare*) essential oils, clove oil not only inhibited the mycelial growth of *Penicillium expansum* and *Penicillium implicatum* at the tested concentrations (25, 50, 100 and 250  $\mu$ L/L, performing maximum at 250  $\mu$ L/L giving 100% fungal growth inhibition), but also showed 64 and 45% disease reduction against *P. expansum* and *P. implicatum* respectively, when applied as curative treatment to the artificially inoculated fruits ( $1 \times 10^6$  spores/mL). In addition, Clove oil treatment also reduced weight loss and maintained soluble solid contents, titratable acidity and ascorbic acid contents of pomegranate fruits for about 4 weeks at ambient temperature (25°C). Hence, it is concluded that clove oil can be used for postharvest disease management, preservation and extension of shelf life of pomegranate fruits.

#### Summary of 11-year field trials of using Heads Up® - a Saponin plant protectant to manage soybean diseases

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Heads Up® is a plant protectant that induces systemic acquired resistance of plants when applied as seed treatments or foliar sprays. Since 2005, field trials have been established in multi-locations of Iowa State University Research Farms to determine effectiveness of seed treatment to manage soybean

sudden death syndrome and white mold. Eleven-year Iowa data together with some data from other mid-west soybean production states were pooled and analyzed. Results show that in average seed treatments with Heads Up alone or Heads Up plus other compound improved soybean yields 2.08 bushels per acre over untreated control. Seed treatments effectively reduced or suppressed the occurrence of SDS and white mold in soybean. Compared with control, the probabilities of yield response to a treatment were 63.5%, 7%, 29.5% for increase, no change, and decrease, respectively. Compared with synthetic chemicals, there is a slightly larger number of low responsive trials for Heads Up, which is often seen in field trials with biological control products. However, the low cost (<\$2 per acre) and its biological nature makes Heads Up a good candidate for soybean disease management. To improve consistency, factors leading to low responsiveness are investigated and addressed.

#### **Efficacy of synthetic and biorational products against powdery mildew of flowering dogwood**

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Flowering dogwood (*Cornus florida* L.) is a popular ornamental plant in home gardens and commercial settings. Powdery mildew, caused by *Erysiphe pulchra*, may cause cosmetic damage on flowering dogwood by attacking tender shoots and leaf surfaces that cause for dead patches and premature defoliation or kill seedlings. Various products are available or in development that have the potential to contribute to the management of powdery mildew of dogwood. In this study the effect of biorational products (Triact 70-neem oil, thymol and cedar oil) and fungicide (Cleary's 3336F) on dogwood powdery mildew severity was evaluated. Treatments were applied using hand-held sprayer every 7 days for a total of five applications. The experiments were done twice, each with three replicates per treatment. The severity of powdery mildew on foliage and phytotoxicity were evaluated using a scale of 0-100% foliage affected. In both experiments, the severity of powdery mildew was low to moderate (~15 and 30% foliage affected-non-treated control). Triact 70, thymol and Cleary's 3336F provided significant control of powdery mildew compared to the non-treated control in both experiments. Powdery mildew disease progress was lower in dogwood plants treated with Triact 70, thymol, Cleary's 3336F and the high rate of cedar oil than in those non-treated control and the low rate of cedar oil. However, thymol and the high rate of cedar oil were slightly phytotoxic on dogwood foliage.

#### **Antifungal effect of nano sized zero valent iron (nZVI) to special fungal pathogens in paprika and tomato crop**

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Zero-valent iron (ZVI) has been utilized in environmental remediation including the removal of pollution or contaminants such as heavy metals, phosphate as an ingredient of fertilizers and pesticides in groundwater, soil and/or sediment. Recently, many studies have shown that the nano-sized ZVI (nZVI) have antibacterial and antifungal activity by oxidative reaction of the nZVI. Here, we are trying to apply the ZVI or nZVI as antifungal agent instead biocidal chemical compound (fungicide) for *Collectorichum acutatum*, *Fusarium oxysporum* f. sp. *Lycopersici*, *Sclerotinia minor*, *S. sclerotiorum*, and *Rhizoctonia solina*, the causal agent of major fungal disease of paprika and tomato crop in Korea. The result of antifungal activity tests showed that ZVI and nZVI were the most efficient antifungal agent to all five fungal pathogens. Especially, nZVI was more efficient to inhibit the hyphal growth than ZVI in five fungal pathogens.

#### **Natural products reduce grey mold of tomato caused by *Botrytis cinerea***

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Grey mold is the most important postharvest disease of tomatoes in Hawaii. Treatments with edible, natural products are needed to reduce losses and contribute to food sustainability. Based on the hypothesis that inhibition of *Botrytis* spore germination will significantly reduce postharvest losses, botanicals were tested for their effects on conidia. Ten *Botrytis* isolates from rotting tomato fruit were identified morphologically and confirmed by DNA sequence analysis. Leaves of candidate plants were frozen at -20°C and plant fluids were sterilized by passing through a 0.22 µm millipore. Extracts were evaluated by adding *Botrytis* spores (10<sup>6</sup>/ml) with sterilized malt extract broth to multi-well microplates. Changes in optical density measured at 6, 12, and 24 hours after inoculation were analyzed with Gen5 software. *Capsicum chinense* cultivars Datil and *C. annuum* Carnival completely inhibited fungal germination in preparations containing 30 and 40% leaf extract at all evaluation times. These extracts were superior to all other extracts tested. Extracts of *Waltheria indica* and *C. frutescens* had intermediate antifungal activity. *Aloe vera*, *Tagetes patula* and *Capsicum annuum* cultivars Red pepper, Criolla de cocina, New Mexico, showed no measurable antifungal activity. Extracts from the two most promising pepper cultivars, Datil and Carnival, are being evaluated as pre-harvest sprays in the greenhouse and as edible coatings on tomato fruit postharvest to reduce grey mold.

#### **Identification of volatiles with nematocidal activities from *Sphingobacterium nematocida* ZY-71-1 and control efficiency to *Meloidogyne incognita***

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Chemical pesticide is an effective way in controlling tobacco disease, but it caused some problems such as environment pollution, ecological balance destruction and chemical residues. So biology control of tobacco disease attracts more and more attention. The usage of micro-organisms or microbial metabolites is an important approach in controlling nematodes. *Sphingobacterium nematocida* ZY-71-1, a bacterial endophyte, was isolated from tobacco leaves, which producing volatiles with nematocidal activities. The biocontrol effect of the bacterium towards *Meloidogyne incognita* was evaluated under Petri dish test and greenhouse assay. The mortality rate to the nematode caused by *S. nematocida* ZY-71-1 was 100% and the inhibition rate to the hatch of the eggs of the nematode was also 100%. Additionally, nematocidal bioactivity materials produced by *S. nematocida* ZY-71-1 were determined. By GC/MS analysis and pure chemicals confirmation, 5 volatiles with nematocidal activities against juveniles and eggs of *M. incognita* were identified from *S. nematocida* ZY-71-1, including benzeneacetaldehyde, 2-nonanone, decanal, 2-undecanone and dimethyl disulfide. This study provided an effective way for biological control of nematode and established foundation on controlling the nematode.

#### **The Role of the Citrus Microbiome in Tree Health and Tolerance to Pathogens**

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There is compelling evidence that the health of an animal or plant is linked to the structure and activities of its associated microbiome. The goal of this project is to identify naturally occurring microbes or consortia of microbes that either directly or indirectly protect citrus trees from plant pathogens. We have, thus far, isolated and identified the culturable microbes of the roots, leaves, and budwood of citrus trees collected from California and Florida. We isolated both fungi and bacteria, and sequenced the amplified 16S rRNA gene from bacteria and the ITS region of the ribosomal operon from fungi for microbial identification. A culture-independent analysis of the citrus microbiome using an Illumina MiSeq platform has also allowed us to reveal a more comprehensive view of the microbial communities that inhabit citrus. We hypothesize that the citrus microbiome and their associated metabolomes can be used to reduce or prevent colonization of citrus pathogens, such as *Candidatus Liberibacter asiaticus* (CLAs). We are currently screening compounds produced by these isolated citrus microbes for inhibitory properties against CLAs. We envision the discovery of bactericide producing microbes that

could be directly transferred and established on trees, or the naturally produced bactericide could be purified and applied to combat *Clas* and/or other pathogens.

#### **Fungal antagonism of bacteria isolated from Cultivated and Wild Cranberry Bogs**

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Natural environments are characterized by a relative equilibrium that can be brought into disequilibrium by the introduction of agricultural plants. Both natural and agronomic systems should thus contain microbes that contribute to system equilibria by suppressing parasitic organisms. However, few biological niches have been systematically explored for biological control agents. Cranberry bogs in southeastern Massachusetts are a good model system for these studies because of the genetic similarity between wild and domesticated cranberry plants, and their location in the same geographic region. Soil and plant samples were collected from wild and cultivated bogs over several growing seasons to determine the microbial composition of cranberry bogs. 68 bacterial isolates were assessed for *in vitro* inhibition of the pathogenic fungi, *Phomopsis* sp., *Colletotrichum* sp., and *Coleophoma* sp. isolated from cranberry, as well as *Trichoderma* sp. Individual isolates varied in antifungal ability within and between fungi. *Bacillus* spp. were most antagonistic to these fungi, but several other bacterial genera were active against one or more of the fungi as well. In a preliminary assessment, 82% of the chosen bacterial isolates had activity against *Phomopsis*, 44% against *Colletotrichum*, 63% against *Coleophoma*, and 19% against *Trichoderma*. These results suggest that bog soils and plants harbor a large number of candidates for development as biological control agents in agriculture.

#### **Relative efficacy of Zambian atoxigenic *Aspergillus flavus* strains against aflatoxin-producing *Aspergillus* species**

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In Zambia, fields cropped to maize and groundnuts are predominantly colonized by aflatoxin producing *Aspergillus flavus* S-morphotypes and *A. parasiticus*. Consequently, Zambian staple foods are often contaminated with high aflatoxin levels (>20 ppb) while in the field and during storage. This study aimed at identifying the best atoxigenic *A. flavus* L-strains for use as biocontrol agents. *In vitro*, the most toxigenic L (25) and SBG (10) strains were selected out of the entire isolate library based on qualitative and quantitative TLC assessment. Competition assays were performed by co-inoculating 14 atoxigenic candidate strains and the 35 selected toxigenic ones on sterile maize grains in sterile vials. All the 14 atoxigenic strains outcompeted the toxigenic ones and reduced aflatoxin production by >96% *in vitro*. Eight of the 14 atoxigenic strains were selected and formulated into two mixes, with each mix comprising of 4 strains. Each mix was applied to maize and groundnut fields between 2012 and 2015 at a rate of 10 kg/ha, 2-3 weeks before flowering. Treating fields with the two mixes altered *Aspergillus* soil community structure and significantly reduced aflatoxin levels in maize and groundnuts by 89.6% compared to untreated ones. Thus, the 8 atoxigenic strains hold promise for fighting aflatoxin burden in maize and groundnut. Adopting such strains to address aflatoxin could improve grain nutritional quality and safety for better health of the Zambian populace.

#### **Incidence of soybean frogeye leaf spot in Korea and selection for antagonistic bacteria controlling *Cercospora sojina* Hara**

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Soybean frogeye leaf spot caused by the fungus *Cercospora sojina* Hara, has been seen a steady increase in the incidence and prevalence on soybean in Korea. The nationwide survey of the occurrence of frogeye leaf spot showed that 11 out of 28 regions in 2015 had been infected with *Cercospora sojina* Hara. To control the disease, an isolate which showed strong inhibitory effect on the mycelial growth and conidial germination of *Cercospora sojina* Hara was selected among the antagonistic bacterial isolates collected from soybean grown soil and the bacterial isolate was identified as *Paenibacillus polymyxa* using 16S rRNA sequence analysis. *Bacillus subtilis* QST713(Serenade) was also inhibitory effect on the mycelial growth and conidial germination. The inhibition rates of these antagonistic bacteria were 53%, 75%, respectively. They could be effectively used for controlling soybean frogeye leaf spot.

#### **Integration between 2,4-DAPG-producing *Pseudomonas* and fluquinconazole to control take-all disease of wheat in Chile**

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Take-all disease of wheat, caused by the fungus *Gaeumannomyces graminis* var. *tritici* (Ggt) could be controlled by integrating fungicide seed treatments with 2,4-Diacetylphloroglucinol-producing *Pseudomonas* spp. (2,4-DAPG). This research assessed the effect on yield parameters and disease damage associated with the integrated use of seed treatments with two Chilean 2,4-DAPG-producing *Pseudomonas* spp. ( $10^6$  ufc/g grain seeds) and the triazole fungicide fluquinconazole (1.8 mL/g grain seeds). A field experiment was established in Chillán, Chile, during crop season 2015. Experimental field plots of 2.4 m<sup>2</sup> with winter wheat cv. Maxwell were seeded to assess combinations with or without the mentioned seed treatments, plus two untreated control (infested or non-infested with Ggt) on a randomized block design. Seed treatments where both strains of *Pseudomonas* were mixed with the fungicide reduced plant and root rot disease severity damage (50 and 49%, respectively) and white heads (32%), while increased yield (34%) and grain quality (2.1%) with respect to the Ggt infested control ( $P \leq 0.05$ ). Single use of bacterial seed treatments without the fungicide was not different from this control ( $P \geq 0.05$ ). Fluquinconazole alone had a lower grain yield (13%) and wheat test weight (1.4%) than when it was mixed with the two bacterial strains. Results suggest that integration of 2,4-DAPG-producing *Pseudomonas* with fungicides could reduce take-all disease and increase yield in wheat crops.

#### **Stopping the cereal killer: Exploring biological control to mitigate Fusarium head blight of wheat**

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Fusarium head blight (FHB) is a devastating fungal disease impacting wheat and other cereal production globally. Exacerbating the impact of disease is the *in planta* production of the toxic fungal secondary metabolite and virulence factor, deoxynivalenol (DON). DON is an inhibitor of the eukaryotic ribosome thereby blocking protein translation, and in toxicological studies has been found to affect the gastrointestinal, immune, and endocrine systems of humans and animals. Preventing DON accumulation in grain ultimately begins with preventing infection by FHB causing *Fusarium* species. Current mitigation strategies to combat FHB include the use of moderately resistant varieties of wheat, crop rotations with non-hosts, and fungicides, but even in conjunction these strategies provide only minimal to moderate control when environmental conditions are conducive to disease. This research aims to identify and characterize biological control agents to mitigate FHB through media based screening of samples from different production systems and geographic locations for *Fusarium graminearum* inhibiting and DON degrading microbes. Exploring multiple sources including various soils, plant material, silage, and haylage will allow for a diverse range of microbes exhibiting the aforementioned capabilities to be studied for their specific mechanisms, potential common genetic basis, and potential use in microbial consortia for disease control.

### Characterization and distribution of natural populations of *Cryptococcus flavescens* across the United States

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Fusarium head blight (FHB) is an important disease of small grains caused primarily by *Fusarium graminearum*. This disease causes grain yield and quality losses under conducive conditions. The yeast *Cryptococcus flavescens* is an effective biological control agent of FHB on wheat. In this study, the biogeography and genetic diversity of natural populations of *C. flavescens* across the United States were characterized. Wheat spikes samples were collected at flowering and at harvest and assayed for *C. flavescens* using specific PCR primers designed on the heat shock protein *hps70KDa*. This assay is 1,000 times more selective for *C. flavescens* than other *Cryptococcus spp.* Samples with > 106 cells/g of wheat tissue were subjected to microbial isolation, and colonies with *C. flavescens*-like morphology were selected and characterized using amplified ribosomal DNA restriction analysis (ARDRA) profiling and multilocus genotyping. The results showed that *C. flavescens* was widespread across the country, albeit at low incidence (approximately 10%, n = 320). Spikes were the preferred tissue of colonization. When present, populations of *C. flavescens* ranged from 105-107 cells/g of tissue. Plant growth stage influenced *C. flavescens* colonization. This work confirms that *C. flavescens* is found across a broad geographical area in close association with wheat. Further, it demonstrates that *C. flavescens* is a non-invasive biological control option for FHB in the regions sampled.

### Biocontrol of Fusarium head blight with *Bacillus spp.*

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Fusarium head blight, caused by *Fusarium graminearum*, has become a cause of concern in many portions of Alabama. This disease was severe in Southern AL, due to frequent rainfall during flowering in 2015. Infection of *F. graminearum* reduces quantity and quality of yield by infecting the kernels of many grain crops; it also produces the mycotoxin, deoxynivalenol (DON). Wheat grain was collected from fungicide treated plots and found to be infested with *Fusarium spp.* Cultures were established from single spores and species were determined from morphology of spores and color of the fungus when mature. Biocontrol potential of selected *Bacillus* strains against *F. graminearum* was assayed using a dual culture method. Based on inhibition of fungal growth and spore production, strains were selected that were most suppressive against *F. graminearum*. In order to test the effectiveness of these strains in the field, five *Bacillus* strains will be applied to wheat heads during flower, and disease intensity will be rated 3 weeks later. Results of the field study will be reported. Any reduction in Fusarium head blight by a biological agent might contribute to overall management of this disease.

### Selection and characterization of probiotics for rye silage

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Silage is feed of livestock. Forage crops are at risk from infection by mycotoxins producing fungi. Rye has considerable as a highly quality of forage. After heading stage, the biomass of rye increased up to 30%. However, lignification caused low fermentation efficiency and coefficient digestibility. This study was performed to develop probiotics which improve quality of silage in nutrient aspect. During the fermentation period of the rye silage, dominant microorganisms were selected. Those were confirmed antimicrobial activities against fungi and were tested acidification ability. The isolates confirmed ability of fibrinolysis through the four enzyme (cellulase, xylanase, esterase, chitinase). The final selected strains were added to the rye silage and then confirmed the effect by real-time PCR in antifungal activity. Total 180 isolates were isolated from the rye silages during various fermentation period. Among 180, 8 isolates showed antimicrobial activities against *Fusarium moniliforme*. two isolates among antimicrobial activities isolates showed strong acidification ability. Also a single isolate was selected that was effective all four enzymes. In real-time PCR results, the mycotoxin gene was detected significantly low in the rye silage, which added antimicrobial activity isolate. In case developed microorganism product was applied at other stock feed as well as rye silage, production of high quality forage will expand.

### Inhibition of *Penicillium expansum* growth on Golden Delicious apples by wild yeasts from various plant materials

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Wild yeast strains previously isolated from various fruits were tested for their ability to inhibit *Penicillium expansum in vivo* (on Golden Delicious apples) in an effort to determine their potential as biocontrol agents (BCAs). Tests were performed at room temperature (~21°C) for up to 2 weeks and at refrigeration (3°C) for up to 2 months. *Candida oleophila* was used as positive BCA control. All strains tested showed some degree of biocontrol activity against *P. expansum*. At room temperature, activity (lesion size reduction) ranged between 37 and 50% during the first week. After 14 days of incubation, decays on yeast-treated apples were slightly smaller (16-19%) than those on apples treated with *P. expansum* alone. At refrigeration, lesion reduction ranged between 54 and 76% in the first month of storage and between 60 and 76% after 2 months of incubation. Decay incidence was 100% in apples stored at room temperature and 25-62% in those kept under refrigeration. The inhibitory activities of the wild yeasts were similar to those exhibited by *C. oleophila*. These strains showed potential as BCAs against *P. expansum* on stored apples.

### Evaluation of potential biocontrol agents against fungal plant pathogens affecting strawberry

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*Botrytis cinerea*, *Fusarium oxysporum* f. sp. *fragariae* and *Macrophomina phaseolina* are important pathogens that limit commercial strawberry production in California. The objective of this study was to evaluate eight species of bacteria (19 strains) for their ability to inhibit mycelial growth of all three pathogens *in vitro*. Percent inhibition of fungal growth due to the presence of each bacterium was determined relative to growth in the absence of each bacterium. Three strains of *Bacillus amyloliquefaciens* provided the greatest inhibition of *B. cinerea*, *F. oxysporum* f. sp. *fragariae* and *M. phaseolina* by an average of 43.7%, 47.8%, 42.7%, respectively, between replications. Significant differences in fungal inhibition were observed between strains of the same bacterial species, suggesting inhibition is strain-dependent. Growth inhibition caused by five *B. subtilis* strains varied from 39.5% to 0.6% for *B. cinerea*, 43.1% to 0.6% for *F. oxysporum* f. sp. *fragariae*, and 39.8% to 5.8% for *M. phaseolina*. Four *B. licheniformis* strains also displayed similar levels of strain-dependent inhibition. Bacterial antagonists that limit the growth of these soilborne and foliar pathogens could provide an alternative to the use of chemical fumigants and fungicides, or could be utilized to enhance the effectiveness of conventional or organic disease control treatments such as anaerobic soil disinfestation.

### Effects of *Clonostachys* species on charcoal rot disease caused by *Macrophomina phaseolina* in soybean

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Charcoal rot disease caused by *Macrophomina phaseolina* in soybean greatly disrupt stand establishment and cause high yield losses. The pathogen was estimated to cause yield losses of over 270,000 bushels in Nebraska (NE) in 2011. Chemicals are used against many diseases but not consistently effective in the management of soilborne diseases such as charcoal rot. In 2014 and 2015, surveys were conducted in soybean fields in NE. Infected

samples were collected, plated onto potato dextrose agar amended with 0.01% tetracycline (PDA-tet) and incubated in the dark for 4 days at 25°C. Morphology was examined and genomic DNA was extracted from isolates using MoBio microbial isolation kits. Two genes of interest, ITS and  $\beta$ -tubulin, were PCR-amplified. Amplicons were sequenced at the University of California Riverside Gencore. The pathogen *M. phaseolina* was identified but different species of *Clonostachys* (anamorph: *Bionectria* spp.), including isolates identical to *C. rhizophaga*, *C. byssicola*, and *C. rosea* were also recovered from the samples. It appeared from the field samples that *Clonostachys* spp. had negative impacts on the population of *M. phaseolina*. In repeated *in vitro* assay as well as *in vivo* co-inoculation through Rolled Towel Assays indicated suppression of *M. phaseolina* by *Clonostachys* spp. There is need for further studies to better understand the biocontrol efficacy of each *Clonostachys* sp. as well as the mechanisms involved.

#### **Trichoderma asperellum survival and parasitism of Sclerotinia sclerotiorum sclerotia in different soil types**

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Effectiveness of bioagent *Trichoderma asperellum* is influenced by physical, chemical, biological and environmental factors, but there is no clear idea of how such variables affect antagonist endurance. This study assessed the survival of *T. asperellum* and the parasitism of *Sclerotinia sclerotiorum* sclerotia in different Brazilian soils. The test was carried out in a completely randomized design, arranged in a 4 × 6 factorial schedule with five repetitions. Experimental factors comprised six soils of distinct texture and fertility profiles, and four biocontrol treatments with three *T. asperellum* isolates and a water control. Plots consisted of 12cm x 22cm x 3cm transparent plastic containers, with sclerotia distributed over 300g of soil. After spraying with  $2.0 \times 10^{12}$  *T. asperellum* conidia ha<sup>-1</sup> and soil moisture adjusted to 100% field capacity, treatments were incubated at 20°C with 12h photoperiod for 28 days. Weakly assessments considered sclerotia parasitism, area under antagonist survival curve (AUASC), culturable fungi and bacteria, and bacterial diversity estimated with Biolog Ecoplates. Principal component analysis showed that parasitism of *S. sclerotiorum* sclerotia was proportional to *T. asperellum* AUASC. In contrast, antagonist survival was negatively affected by soil pH, bacterial density and diversity, organic matter, Ca, Mg and K contents. Biocontrol efficiency varied according to soil source.

#### **In-vitro control of Calonectria pseudonaviculata by bacteria recovered from irrigation water**

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*Calonectria pseudonaviculata* (*Cps*) is the causal agent of boxwood blight. This disease first observed in USA in 2011 has significantly impacted the nursery and landscape industries. The objective of this study was to develop biologically-based, long-term solutions to managing this emerging disease at production facilities and in the landscapes. Over 1000 bacterial strains recovered from irrigation water in Virginia, USA were screened for their potential as biological control agents against microsclerotia of *Cps*. Microsclerotia were harvested from potato dextrose agar cultures and plated on nutrient agar in 48-well plates. Two days later, fresh bacterial cultures grown in nutrient broth were added to the culture of *Cps*. Three replicate wells per bacterial strain were used. Sterile nutrient broth was added to three wells per plate as controls. Microsclerotia germination and resultant mycelial growth was measured based on a 0-to-5 rating scale at five and ten days after adding bacterial cultures. It was found that approximately 30% of screened bacterial strains greatly suppressed microsclerotial germination and mycelial growth with average ratings of one or less. These bacteria are promising biological control agents (BCAs) for boxwood blight and warrant further evaluation using detached leaf assay, greenhouse and field *in-vivo* tests.

#### **Characterization and distribution of natural populations of Cryptococcus flavescens across the United States**

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Fusarium head blight (FHB) is an important disease of small grains caused primarily by *Fusarium graminearum*. This disease causes grain yield and quality losses under conducive conditions. The yeast *Cryptococcus flavescens* is an effective biological control agent of FHB on wheat. In this study, the biogeography and genetic diversity of natural populations of *C. flavescens* across the United States were characterized. Wheat spikes samples were collected at flowering and at harvest and assayed for *C. flavescens* using specific PCR primers designed on the heat shock protein hps70KDa. This assay is 1,000 times more selective for *C. flavescens* than other *Cryptococcus* spp. Samples with > 10<sup>6</sup> cells/g of wheat tissue were subjected to microbial isolation, and colonies with *C. flavescens*-like morphology were selected and characterized using amplified ribosomal DNA restriction analysis (ARDRA) profiling and multilocus genotyping. The results showed that *C. flavescens* was widespread across the country, albeit at low incidence (approximately 10%, n = 320). Spikes were the preferred tissue of colonization. When present, populations of *C. flavescens* ranged from 10<sup>5</sup>-10<sup>7</sup> cells/g of tissue. Plant growth stage influenced *C. flavescens* colonization. This work confirms that *C. flavescens* is found across a broad geographical area in close association with wheat. Further, it demonstrates that *C. flavescens* is a non-invasive biological control option for FHB in the regions sampled.

#### **Curvularia prsadii as a biological control agent for barnyard grass (Echinochloa crus-galli) in rice fields**

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*Echinochloa crus-galli* is one of the most important damaging weeds of rice paddies. Three isolates of *Curvularia prsadii* (CP01, CP02, and CP03) were isolated from the rice weed, *E. crus-galli* in three governorates (Dakahlia, Kafr El-Sheik, and El-Garbeia) in Egypt in three consecutive growing seasons (from 2012 to 2014). The biocontrol activity of these fungal isolates was determined on the weed seedlings in the greenhouse using spore suspension of 10<sup>6</sup> conidia/ml. All of the three isolates tested produced 40-60% disease severity on the weed seedlings with no significant differences among them. Host range studies were conducted to determine the host specificity and safety of the three isolates of the bio agent, *C. prsadii* against 23 non-target plant species and cultivars of economic importance grown in Egypt. No disease symptoms appeared on any of the non-target plants tested. The optimum nutritional and physical conditions for the production of the biocontrol agents were determined and will be discussed.

#### **Biocontrol of Tubakia leaf blight by Paenibacillus peoriae(HB774)**

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South Korea has shown decreasing proportion of coniferous plants and increasing proportion of deciduous plants due to the effect of global warming. With the growing significance of deciduous forests, the national forest damage by ongoing Tubakia leaf blight of the oak is more severe than before. In response to the current critical forest situation of Korea, this study aims to develop the efficient biocontrol agent of the Tubakia leaf blight. Among 30 bacteria isolated from soil and plants, HB774 showed the highest inhibition rate (85.8%) of the fungal mycelial growth, which was selected as a potentially effective biocontrol agent. As a result of the 16S ribosomal DNA sequence analysis, a gram-positive bacterial isolate was identified as *Paenibacillus peoriae* and deposited as a patent strain in Rural Development Administration of Korea. This isolate inhibited the mycelial growth by 69.7% and 75.6% at the bacterial concentrations of  $1 \times 10^6$  CFU/ml and  $1 \times 10^8$  CFU/ml, respectively, of which the inhibitory rates were identical to or significantly higher than those of the fungicides, propiconazole and iminonctadine-triacetate. In the simultaneous treatment and pre-treatment of HB774, its control efficacies were high with 80-90%, showing no significant differences from those of propiconazole and iminonctadine-triacetate. This suggests *P. peoriae* HB774 has a high potential to be developed as an efficient biocontrol agent against the Tubakia leaf blight on oaks.

### **Acaromyces ingoldii inhibits the laurel wilt pathogen, *Raffaelea lauricola* in vitro**

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Laurel Wilt is a destructive disease of redbay (*Persea borbonia*) and other species in the laurel family (Lauraceae) in the southeastern United States (US). The disease is caused by *Raffaelea lauricola*, a fungal symbiont of the redbay ambrosia beetle (*Xyleborus glabratus*). The beetle and the fungus were introduced into the US probably with wood packing materials. This investigation was prompted by antifungal activities demonstrated by an *Acaromyces* species isolated from a loblolly pine bolt in Louisiana during routine laboratory microbial isolations. Our objective was to evaluate the inhibitory property of the *Acaromyces* species against *R. lauricola*. We obtained isolates of *R. lauricola* from fresh laurel wilt infected tissues collected from sassafras trees in Louisiana and Texas, and evaluated the competitive ability of the *Acaromyces* and the fungistatic activities of secondary metabolites produced by the fungus against *R. lauricola* in vitro. Results showed a strong inhibition of *R. lauricola* mycelia growth by the *Acaromyces* species. Growth inhibition was significant as pigmented secondary metabolites produced by *Acaromyces* diffuses through the media. Only minimal direct competition was observed between the two fungi. *R. lauricola* isolates inoculated 7-day after *Acaromyces*, showed no growth and no spore germination. Future experiments will examine the identity, efficacy, and potential benefits of secondary metabolite(s) produced by the *Acaromyces* species.

### **Applications of *Bacillus amyloliquefaciens* PMB01 for managing Fusarium wilt of cucumber**

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Cucumber Fusarium wilt, caused by *Fusarium oxysporum* f. sp. *cucumerinum* (*Foc*), is the most devastating disease on cucumber. Currently, no effective chemicals are available for disease control; however, prior studies showed that antagonistic rhizobacteria can reduce the disease severity, suggesting that biocontrol agents might be potential solutions for the management of Fusarium wilt disease. In this study, soil bacteria with inhibitory activity on in-vitro *Foc* growth were isolated. Among them, *Bacillus amyloliquefaciens* PMB01 showed the best antagonistic activity against *Foc* in confrontation assays. The efficacy of PMB01 on controlling Fusarium wilt disease of cucumber (FWC) was evaluated in field experiments, and the results showed that applications of the formulated PMB01 reduced the disease severity of FWC down to less than 15%. Prior research also revealed that the formulated PMB01 effectively controlled bacterial wilt disease of tomato (BWT), caused by *Ralstonia solanacearum*, in field trials. Whole genome shotgun sequencing showed that PMB01 harbors genes involved in the biosynthesis of cyclic lipopeptides, bacillibactin, and bacilysin, which might collectively contribute to its biocontrol activity. Toxicology testing demonstrated that PMB01 was non-toxic to mammals. In 2016, the biopesticide Saviour® containing *B. amyloliquefaciens* PMB01 as the active ingredient was developed and commercialized in Taiwan for integrated management of BWT and FWC.

### **The potential use of a *Cladospirium* sp. as a biological control agent for white mold caused by *Sclerotinia sclerotiorum***

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*Sclerotinia sclerotiorum* (Lib.) de Bary is a pathogenic ascomycete known to cause disease in more than 400 plant species, including white mold in potato. In this study, a fungal contaminant found associated with *S. sclerotiorum* that appeared to inhibit *S. sclerotiorum* growth was isolated, sub-cultured, and identified. Morphological characteristics and sequence data from internal transcribed spacers 1 and 2, 5.8s rDNA, and *Actin* and *Calmodulin* genes were consistent with the unknown fungus belonging to the *Cladospirium* genus. A bioassay was used to examine inhibition of *S. sclerotiorum* growth by the *Cladospirium* isolate. A 5mm plug of the *Cladospirium* isolate was plated on potato dextrose agar and left to grow for 72 hours before a 5mm plug of *S. sclerotiorum* was plated 50mm from the *Cladospirium* plug. Isolates of *S. sclerotiorum* and *Cladospirium* were plated alone as controls. The plates ( $n=10$  per treatment) were incubated for 4 days at room temperature with a 12:12 photoperiod. Radial growth of *S. sclerotiorum* mycelia was measured and the experiment was repeated in entirety one time. Averaging over both experiments, the radii of *S. sclerotiorum* plated with the unknown isolate was reduced by 34.1 mm (Paired t-test:  $t = 27.4$ ,  $df = 19$ ,  $p < 0.001$ ) when compared to the radii of the *S. sclerotiorum* plated alone. These data indicate that the *Cladospirium* isolate has potential use as a bio-control agent against *S. sclerotiorum* and should be further evaluated in vivo.

### **Infection duration of entomopathogenic fungi *Aspergillus* spp. and *Fusarium* spp. against *Bemisia tabaci***

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Entomopathogenic fungi are emerging as biocontrol of insects and new fungi are isolated from different insects with passage of time. The aim of present study was to observe the steps of infection of different *Aspergillus* and *Fusarium* species and their duration of infection against *Bemisia tabaci*. To observe the infection duration, spore suspension of different species of *Aspergillus* and *Fusarium* already isolated from insects were used on nymphal stage of whitefly and data regarding duration of spore germination, penetration, colonization and infection was determined under control conditions. It was observed that spores of *Aspergillus* species germinate quickly as compare to *Fusarium* species. There was no significant difference in spore germination on species level. Penetration time of *Aspergillus* and *Fusarium* species vary and colonization and infection duration of *Aspergillus* species was less as compared to *Fusarium* species against nymphal stage of whitefly. In this study, it was observed that *Aspergillus* is more aggressive as compare to *Fusarium* species and also dependent on isolates of insect associated fungi. Further studies required on genetic level that makes entomopathogenic fungi more aggressive on different stages of infection process.

### **Field Performance of Endophytic Actinomycetes in Relation to Plant Growth Promotion and Biological Control of *Fusarium oxysporum* a Pathogen of Tomato**

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Four endophytic actinomycete strains identified as *Actinoplanes missouriensis*, *Streptomyces olivaceiscleroticus*, *S. globosus* and *Dactylosporangium aurantiacum* were obtained in a previous study from surface-disinfested tomato roots. The performance of these four strains was evaluated under field conditions to determine their potential to promote plant growth and to reduce the incidence of wilt disease of tomato caused by *Fusarium oxysporum* f.sp. *lycopersici*. When applied individually or in combination to tomato seedlings, the four strains significantly promoted growth and reduced the incidence of the disease. Individually the performance level of *A. missouriensis* was relatively the best followed by *S. olivaceiscleroticus*, then by *S. globosus* and then by *D. aurantiacum*. The combination of the four strains resulted in significantly better suppression of the disease and growth promotion, compared to individual strains. The ACC deaminase-producing isolates (ACC<sup>+</sup>) (*A. missouriensis* and *S. olivaceiscleroticus*) were significantly more effective in reducing the incidence of the disease compared to ACC deaminase-non-producing (ACC<sup>-</sup>) isolates (*S. globosus* and *D. aurantiacum*). The overall performance of the endophytic strains is promising and could replace the fungicides currently in use for the control of *Fusarium* diseases. The results also showed the potential to enhance the biocontrol agent's performance by including the ACC deaminase ability into the biocontrol strains.



### Assessment of three *Trichoderma* species to protect cabbage from Clubroot

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Following reports that biocontrol agents are potential management tools for clubroot of crucifers, caused by *Plasmodiophora brassicae*, this research aimed at assessing one strain of each of three *Trichoderma* species: *T. asperellum* Th034, *T. brevicompactum* Th201 and *T. koningiopsis* Th003 with different number of applications in terms of disease reduction on 'Delus' cabbage. Experiments were conducted in growth chambers and infested fields. For growth chambers, two concentrations of the pathogen were used, and *Trichoderma* was applied once or twice; disease was measured 60 days after planting. For field experiments *Trichoderma* was applied once, seven or 15 times during the cycle, and evaluations were done quarterly. In growth chambers, disease reduction was observed only for the low pathogen concentration; the highest reduction was observed when *T. koningiopsis* and *T. brevicompactum* were applied once. Disease reduction by *T. asperellum* was proportional to the number of applications. Field results were not as clear cut as those from growth chambers; however, disease reduction was observed with *T. asperellum*, and again this reduction was proportional to the number of applications. In conclusion, *T. asperellum* shows potential for clubroot control when applied frequently, whereas one application of either *T. brevicompactum* or *T. koningiopsis* can be useful in disease management in low pathogen concentrations.

### Evaluation of the biocontrol potential of different *Pseudomonas* species in suppressing Pythium root rot in petunia plugs

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*Pythium spp.*, causing root rot and damping off, affects 300+ hosts including several ornamental plants, and has developed resistance to two widely used fungicides, mefenoxam and propanoic acid. This poses a real threat to greenhouse growers' efficiency in cultivating healthy plants. Ohio State researchers have identified several *Pseudomonas* strains that exhibit biological control potential for disease suppression in many crops, including corn and tomato. One strain of each of the species *P. putida*, *P. brassicacearum*, *P. fluorescens*, *P. poae* and *P. protegens* was tested for the ability to suppress the activity of different *Pythium* species in petunia plugs. Since the production of bacterial metabolites is higher in the transition between logarithmic and stationary phases, our first step included building growth curves of the five strains to identify the appropriate time needed for incubation of the bacterial inoculum prior to application to petunia plugs. Cultures were prepared in LB medium and the OD600 values were recorded using a spectrophotometer every two hours for up to 18 hours. Serial dilution plating at each time point provided correspondent CFUs. 'Carpet White' Petunia seeds were sown in 128-plug trays and immediately treated with a  $10^7$  CFU/mL concentration of each *Pseudomonas* strain. After 14 days, plugs were inoculated with the different *Pythium* species. Responses of disease incidence and severity were evaluated until plugs became shipment-ready.

### Microbiome networks: A systems framework for identifying candidate microbial assemblages for disease management

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Network models of soil and plant microbiomes present new opportunities for enhancing disease management, but also challenges for interpretation. We present a framework for interpreting microbiome networks, illustrating how the observed structure of networks can be used to generate testable hypotheses about candidate microbes affecting plant health. The framework includes four types of network analyses. "General network analysis" identifies candidate taxa for maintaining an existing microbial community. "Host-focused analysis" includes a node representing a plant response such as yield, identifying taxa with direct or indirect links to that node, interpreted as either beneficial or detrimental to plant health. "Pathogen-focused analysis" identifies taxa with direct or indirect links to taxon nodes known *a priori* to represent pathogens, which can be interpreted as agonists and antagonists, respectively. "Disease-focused analysis" identifies key nodes for both plant and pathogen responses. We illustrate the interpretation of network structure with analyses of two microbiomes: the oak phyllosphere and soil associated with the presence or absence of infection by *Rhizoctonia solani*. Such network analyses can be used to further characterize microbial communities and associated conditions involved in the suppression of plant pathogens, the biofertilization of crop plants, and/or the expression of host resistance in crop plants.

### Linking microbial taxa in SDS-suppressive soils of Soybean fields

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Sudden Death Syndrome (SDS) is one of the most important diseases of soybean in the Midwest. SDS is caused by the soilborne fungus *Fusarium virguliforme*. SDS frequently appears in circular to oblong patches in a soybean field and are often found unevenly distributed across the same field, while conditions in the rest of the field may appear to be naturally suppressive to the disease. In the SDS-suppressive areas, soybean plants may be less affected by the pathogen because of the activity of other soil microbes. There is a lack of comprehensive studies that investigate the role of soil inhabiting bacteria and fungi in affecting the development of the SDS. By coupling metabarcoding with paired-end Illumina MiSeq sequencing, we identified key microbial taxa involved in the suppression or promotion of the SDS pathogen. Statistical analyses revealed significant differences in fungal and bacterial community composition between suppressive and conducive soils. A microbial consortia involving members of Actinobacteria, *Fusarium oxysporum*, *Trichoderma*, *Penicillium*, *Metacordyceps*, and *Purpureocillium* were found consistently associated with SDS suppression. In contrast, members of the *Fusarium solani* sp. complex were frequently present in conducive soils.

### Isolation of endophytic bacteria associated with roots from citrus infected with *Phytophthora nicotianae*

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Citrus orchards in Texas are greatly affected by *Phytophthora* foot and root rot. Because the presence of antagonistic bacteria associated with plant roots may play a crucial role in root health and disease development we decided to study the effect of *Phytophthora* infection on endophytic bacterial communities associated with citrus roots. Roots from three *Phytophthora* infected and three uninfected grapefruit trees were randomly selected to isolate culturable bacteria. Roots were collected from soil samples taken from each tree at four quadrants, 1 meter away from the trunk at a depth of 15 centimeters. Serial dilutions of macerated root tissues were plated onto nutrient agar, tryptone yeast, King's B and actinomycetes isolation mediums and incubated at three different temperatures. A significantly higher number of cultivable bacteria were present in roots from infected trees versus uninfected trees. In addition, a total of 140 and 119 morphologically distinct bacterial colonies were recovered and isolated from *Phytophthora* infected and uninfected trees respectively. These initial results indicate that citrus trees infected with *Phytophthora* spp. are more prone to develop bacteria-root associations. Characterization of the bacterial species associated with *Phytophthora* infection will lead to the identification of beneficial or antagonistic bacteria against *Phytophthora* spp.

### Seasonal dynamics of fungal communities in pistachio and almond in California: Implications for aflatoxin biocontrol management

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Interactions between pathogenic and nonpathogenic fungal species in the tree canopy are complex and can determine if disease will manifest in the plant and in other organisms such as honey bees. Seasonal dynamics of fungi were studied in almond and pistachio in California. In an almond orchard, experimental release of the atoxigenic biopesticide *Aspergillus flavus* AF36 to displace toxigenic strains has been conducted for five years. Because *A. flavus* is a pathogen of honey bees, our emphasis was on the presence of *A. flavus* in the flowers and the honey bees that attend them. Fungal species most commonly recovered from developing pistachio fruits were: *Cladosporium herbarum*, *Alternaria alternata*, *Penicillium* spp., *Botrytis cinerea*, *Aspergillus niger*, *A. flavus*, and yeasts. The main seasonal changes were detected in *P. expansum* and *A. niger*, which markedly increased from April to September. Conversely, the *B. cinerea* population significantly decreased during this period. In the almond, the dominant genera were: *Cladosporium*, *Aureobasidium*, *Alternaria*, *Aspergillus*, and *Fusarium*. Surprisingly, the diversity index was greater in the summer for both crops. The incidence of almond flowers with *A. flavus* ranged from 0 to 4.5%, depending on the year of study, and the incidence of honey bees carrying *A. flavus* ranged from 6.5 to 10%. This study helps the understanding of seasonal dynamics of pathogenic and nonpathogenic fungi and the impact of AF36 to pollinator honey bees.

#### **Leaf applications of *Clonostachys rosea* reduce the populations of *Botrytis cinerea* and changes the metabolic profiles of strawberry plants**

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*Clonostachys rosea* is an efficient antagonist of *Botrytis cinerea*. In a research program aiming at the biocontrol of gray mold of strawberry, we studied the effect of *C. rosea* application on both antagonist and *B. cinerea* leaf populations, as well as on plant metabolism at a commercial strawberry field production. The antagonist was applied at 7, 14, or 28-day intervals, *B. cinerea* was naturally inoculated, and the colonization of leaves by both fungi was assessed. The antagonist reduced *B. cinerea* colonization; most significant effects were found with week applications. The reduction in *B. cinerea* colonization was negatively correlated with the increase in *C. rosea*'s at all intervals. The metabolic profiles of treated strawberry plants were also most changed with week applications, and there was a significant increase of compounds related to the Krebs cycle, growth promotion and the activation of plant defense system. In conclusion, with week applications of *C. rosea*, there was a significant reduction on the population of *B. cinerea* in symptomless tissues and a change in the metabolic profile of strawberry plants. Sponsored by CNPq and FAPEMIG.

#### **Reduction of soybean sudden death syndrome (*Fusarium virguliforme*) by seed treatment with *Bradyrhizobium japonicum***

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Soybean sudden death syndrome (SDS), caused by *Fusarium virguliforme*, is a major soil-borne disease in soybean production in America. In North America, epidemics of the disease occurred in years when soybeans were planted early in growing seasons. Previous study by Scherm and Yang (1996) showed that *F. virguliforme* infected soybean in cool soil temperatures. Because infection of symbiont *Bradyrhizobium japonicum* requires minimum temperature of 17.5°C, higher than the minimum for *F. virguliforme* infection, we hypothesize that infections of *F. virguliforme* prior to establishment of *B. japonicum* in soybean affects SDS occurrence in early planting. From 2013 to 2015, experiments were conducted in a greenhouse and fields to determine the effects of seed treatments with *B. japonicum* on the occurrence of soybean sudden death syndrome. Results of greenhouse and field studies showed that seed treatments with *B. japonicum* inoculum reduced SDS foliar symptom expression by 50% and 20% in greenhouse and field, respectively. Increase in soybean nodulation and increase in yield in field experiment were also observed. Our results suggest that seed treatment with *B. japonicum* can reduce SDS foliar symptoms. The mechanisms of the suppressive effects on *F. virguliforme* by *B. japonicum* are under investigation to improve the potential of using this measure for disease control.

#### **The use of fungal biological control agents to manage SDS, charcoal rot, and damping off in soybean**

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Sudden death syndrome (*Fusarium virguliforme*), damping off (*Rhizoctonia solani*), and charcoal rot (*Macrophomina phaseolina*) are three important and detrimental diseases of soybean in soybean growing areas. There is a need for more options to control these pathogens; many of the available management options are costly or limited in their effectiveness. Additionally, many of the current control practices may not be sustainable given their unfavorable impact on the environment. The use of biological control agents (BCAs) provides a more natural alternative for controlling various plant diseases. We have evaluated the effectiveness of multiple BCAs against *F. virguliforme*, *R. solani*, and *M. phaseolina*. We have also investigated the mechanisms the BCAs use to control these pathogens. This was determined through the use of microscopy and the monitoring of the expression of a panel of eight mycoparasitism-induced genes.

#### **Biological control of soilborne diseases in organic potato production using hypovirulent strains of *Rhizoctonia solani***

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Soilborne diseases are persistent problems in potato production and alternative management practices are needed, particularly in organic production, where control options are limited. Selected biocontrol organisms, including two naturally-occurring hypovirulent strains of *Rhizoctonia solani* (Rhs1a1 and Bs69) and a commercially available *Bacillus subtilis* (GB03), were evaluated, both individually and in combination, for control of soilborne diseases of potato under organic production practices over three field seasons in Maine. Overall, multiple biocontrol treatments resulted in modest but significant reductions in all three soilborne diseases observed (black scurf, common scab, and silver scurf), reducing incidence and severity of each by 10-32%. All biocontrol treatments reduced black scurf (by 15-32%), whereas only specific treatments reduced common scab and silver scurf (by 10-25%). Combinations including both a hypovirulent strain and GB03 tended to be the most effective treatments, although generally not significantly better than individual strains. Tuber yield varied somewhat by year, but Rhs1a1 and GB03 treatments, as well as combinations, increased yield by 10 to 24% overall. These results indicate that hypovirulent *Rhizoctonia*, along with other biocontrol organisms, may be useful for reducing certain soilborne diseases and enhancing yield in organic potato production.

#### **Evaluation of Management Practices on the Efficacy of *Pasteuria nishizawae* As a Biocontrol Agent of Soybean Cyst Nematode**

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Soybean Cyst Nematode (*Heterodera glycines*) is the most economically significant pathogen on soybean (*Glycine max*), causing over \$1.5 billion dollars in damages annually. *Pasteuria nishizawae*, an endospore-forming soil bacterium, is an obligate parasite of soybean cyst nematodes (SCN). *P. nishizawae* has the potential to provide season-long control of SCN in the form of a seed treatment called Clariva® Complete. In this study, we evaluated the efficacy of the active ingredient, *P. nishizawae*, for the management of SCN on soybean. The field design was a split-plot, randomized complete block design with sub-sampling for the 2014 and 2015 growing seasons. Each split-plot was randomly assigned a row spacing of 30-inch or 15-inch spacing. Each subplot within the split-plot was randomly assigned one of three seeding rates (75K seeds/acre, 150K seeds/acre and 225K seeds/acre) and one of two seed treatments (Clariva® Complete and CruiserMaxx®/Vibrance®). Results showed no significant interaction between seed treatment and yield, but did show significant interactions between both seeding rate and row spacing with yield. No significant interaction was observed between the

SCN count and the yield. Because the soybean variety used had PI88788 resistance, the Hg-type of the SCN population may not have been able to develop on soybeans with this source of resistance. This may have masked the effects of the seed treatment and prevented accurate evaluation of its efficacy.

#### **Effect of biopesticides on *Phytophthora* Root Rot Disease of Oakleaf Hydrangea**

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*Phytophthora* root rot, caused by *Phytophthora nicotianae*, has been reported to infect almost all hydrangeas especially container-grown oakleaf hydrangea (*Hydrangea quercifolia*). The pathogen can be introduced into the nursery via *Phytophthora*-contaminated container stock or cuttings. Experiments were performed in a greenhouse to determine the efficacy of biopesticides and fungicide against *Phytophthora* root rot in oak leaf hydrangea cvs. Queen of Hearts and Munchkin. Treatments applied were the biopesticides RootShield WP (*Trichoderma harzianum* Rifai strain KRL-AG2) and RootShield Plus (*T. harzianum* Rifai strain T-22 and *T. virens* strain G-41) and fungicide Subdue Maxx (Mefenoxam). All the treatments reduced root rot disease severity compared to the inoculated, non-treated controls in both cultivars. There were no significant differences between the treatments. Root rot disease severity was significantly higher in the cultivar Queen of Hearts than cultivar Munchkin. There was no significant effect of treatment and cultivar on root biomass. This study shows that biopesticides, RootShield WP and RootShield Plus, should be considered to reduce the risk of *Phytophthora* root rot on container-grown oakleaf hydrangeas in the nursery.

#### **Putative biocontrol mechanisms of rice bacterial blight by *Bacillus subtilis* TKS1-1**

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Bacterial blight of rice caused by *Xanthomonas oryzae* pv. *oryzae* is one of the devastating diseases of rice plant. The main objectives of the research are to investigate the potential of using *Bacillus subtilis* TKS1-1 as a control agent of rice bacterial blight and the possible mechanisms involved in disease control. *B. subtilis* TKS1-1 showed antagonistic activity against *X. oryzae* WF6. Application of *B. subtilis* TKS1-1 at the time of or prior to inoculation with *X. oryzae* WF6 was found to suppress rice bacterial blight. Additionally, the lesion length of leaf blight was less by drenching application with *B. subtilis* TKS1-1 compared to the water control. Spray-application of *B. subtilis* TKS1-1 was shown to increase the expression of several plant defense-related genes including *PR1a*, *PAL*, *NPR1*, and *WRKY45* at 16 hours post application compared to the water control. Furthermore, drenching application of strain TKS1-1 7 days prior to inoculation of the pathogen was found to increase the activity of phenylalanine ammonia lyase, peroxidase and polyphenol oxidase at 48 hours post-inoculation in comparison with the water treatment. Application of *B. subtilis* TKS1-1 on rice cultivar TNG67 showed significant promotion of plant growth. The findings suggested that *B. subtilis* TKS1-1 exhibited efficacy in suppression of rice bacterial blight and in growth promotion. The biocontrol potential is partly attributed to the induction of plant defense response by *B. subtilis* TKS1-1.

#### **Amino acids influence antibiotic production by *Pantoea vagans* strain C9-1**

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(1) Oregon State University, U.S.A.; (2) USDA-ARS, Horticultural Crops Research Unit, U.S.A.

*Pantoea vagans* strain C9-1 is an effective biological control agent for fire blight caused by *Erwinia amylovora*. C9-1 is thought to suppress *E. amylovora* on stigmas via resource competition and production of the antibiotics pantocin A (formerly herbicolin O) and herbicolin I. We used a green fluorescent protein reporter construct in C9-1 to monitor the regulation of the promoter of the pantocin A operon (*paaPABC*) on solidified media and in broth culture with a Tecan Infinite M200PRO. In assays on solidified media, inhibition of *E. amylovora* by C9-1 and expression of the *gfp* reporter for pantocin A operon was detected on Neidhardt's modified medium, but not on Luria Bertani (LB) medium. Addition of yeast extract or tryptone (components of LB) to Neidhardt's broth medium suppressed the expression of the pantocin A promoter. We tested the effect of various amino acids (100 mM) added to Neidhardt's broth medium on expression of the *paaPABC* promoter in C9-1. We found that arginine, histidine, or tryptophan reduced expression of the pantocin A promoter compared to expression in non-amended Neidhardt's broth medium, whereas asparagine, aspartic acid, or proline upregulated expression. Asparagine and proline are two major amino acids reported on apple and pear flower stigmas. These results indicate that the chemical environment of stigmas likely would support expression of the *paaPABC* operon and pantocin A biosynthesis by the biological control agent C9-1.

#### **Microscopic analysis of parasitism mode of action by bacterial biological control agents on *Cornus florida* powdery mildew**

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Three bacterial isolates, *Bacillus* (sp.), *Stenotrophomonas* *Serratia* (sp.) were previously identified as naturally occurring biological control agents (BCA) for powdery mildew in *Cornus florida*. The interaction between these BCA's and powdery mildew fungus was studied using scanning electron microscopy (SEM) and detached leaf technique. Four replicates of similar leaves were infected with powdery mildew, sprayed with BCA's, incubated for 3h, 6h, 15h, 48h and 5 days, then fixed overnight using formalin acetic acid alcohol and stored at 4°C until SEM imaging was done. The SEM micrographs showed the three BCA's colonizing powdery mildew spores and hyphae. The BCA's displayed the ability to enter and lyse powdery mildew spores and hyphae; the (*Bacillus*) (sp.) lysed and destroyed mycelial structures completely within 48h; others took longer. The BCA's multiplied on the leaf surface and inside powdery mildew spores and hyphae. The three BCA's did not interfere with other microbes on the leaf surface; this displayed potential specificity to powdery mildew and potential preservation of other naturally occurring microflora. The collapsed conidia and lysed hyphae suggested antifungal compounds that caused structural damage may be involved. These observations confirm that the three naturally occurring BCA's may provide a buffering effect against powdery mildew and a resource for powdery mildew management and reducing conventional fungicides usage.

#### **Investigation of antifungal and herbicide volatile terpenes in *Streptomyces* spp.**

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*Streptomyces* spp. produce various secondary metabolites for interaction with environment. Some of these secondary metabolites have been reported as antibiotics or volatile compounds. In addition, *Streptomyces* spp. have been highly expectation for discovering new secondary metabolites. We observed that mycelia of various fungi were inhibited growth when these fungi were exposed to headspace of *Streptomyces* spp. Furthermore, germination and growth of *Arabidopsis thaliana* Col-2 were inhibited significantly by these headspaces. In these headspaces, three volatile terpenes that geosmin, caryolan-1-ol and unknown terpene were discovered in common. To confirm the bioactivity, we extracted mixtures from headspace of *Streptomyces griseus* S4-7. Mixture included geosmin didn't inhibit mycelia growth of *Botrytis cinera*. A mixture included caryolan-1-ol and unknown terpene inhibited these mycelia. Furthermore, synthetic caryolan-1-ol caused dose-dependent inhibition of these mycelia and *Saccharomyces cerevisiae* growth. In addition, we found three terpene cyclase gene in *S. griseus* S4-7 genome. Geosmin and caryolan-1-ol synthases already had been reported but the other terpene synthase was unknown. Our present results show that caryolan-1-ol was discovered new bioactivity function.

### **Characterization and Expression of the Potent Antimicrobial Peptide LsGRP1C Originated from *Lilium***

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(1) National Taiwan University, Taiwan; (2) National Taiwan University, Taiwan

*LsGRP1* is a plant defense-related gene increasingly expressed in lily leaves exhibiting salicylic acid-induced systemic resistance against pathogenic fungus *Botrytis elliptica*. *In vitro* antimicrobial assay applied with synthetic peptides revealed that the 38-a.a. cysteine-rich C-terminal portion (LsGRP1<sup>C</sup>) of LsGRP1 inhibited kinds of bacterial and fungal species *via* the mechanisms of alteration of microbial membrane permeability and induction of fungal programmed cell death (PCD) as investigated by SYTOX Green staining and 4',6'-diamidino-2-phenylindole staining/terminal deoxynucleotidyl transferase dUTP nick end labeling assays. Then the expression system for recombinant LsGRP1<sup>C</sup> production was established using an optimized *Escherichia coli* strain and an N-terminal fusion partner of small ubiquitin-like modifier protein (SUMO) Smt3 originated from yeast, conducting a high yield of SUMO-LsGRP1<sup>C</sup> fusion protein. According to the half inhibitory concentrations for spore germination and PCD occurrence of the assayed fungal species, SUMO-LsGRP1<sup>C</sup> was proven to exhibit higher antifungal activity and identical fungal PCD-inducibility as compared with synthetic LsGRP1<sup>C</sup>. Thus, we proposed that LsGRP1<sup>C</sup> would play a role in antimicrobial function of LsGRP1; moreover, efficient and effective *E. coli* expression strategy would enable the convenient production of antimicrobial LsGRP1<sup>C</sup> for practical use.

### **Functional analyses of the ribosomal and non-ribosomal processed antimicrobial peptides produced by biocontrol bacterium *Bacillus pumilus* PMB102**

W. DENG (1), J. Wu (1), J. Huang (1)

(1) National Chung Hsing University, Taiwan

Many *Bacillus* spp. are beneficial bacteria with potential applications in the integrated management of plant diseases, and the efficacy of biocontrol is mainly determined by the production of secondary metabolites, e. g. the ribosomal processed lantipeptides and non-ribosomal processed cyclic lipopeptides (CLPs). In this study, the biocontrol agent *B. pumilus* PMB102 was shown to reduce tomato Fusarium wilt disease in hydroponic seedling assays. Annotation of the draft genome identified one lantipeptide and two CLPs, surfactin and plipastatin (fengycin family), biosynthetic gene clusters. Partially purified proteins from the lantipeptide-containing culture filtrate were resolved by electrophoresis and assayed for antibacterial activity by overlay assays, and one small polypeptide was identified to inhibit the growth of *B. cereus* and *B. mycoides*. The culture filtrates containing CLPs were extracted by n-butanol and fractionated by C18 SPE column. The eluted fractions were resolved by TLC and assayed for antifungal activity by the overlay assays to show that the compounds sharing the same Rf value of fengycin could inhibit the mycelial growth of *Alternaria brassicicola*. The chemical compositions of the abovementioned antimicrobial compounds will be further verified by mass spectrometry. The expression of defense-associated genes will also be monitored to determine if the plant defense responses against fungal pathogens are modulated by the biocontrol bacterium PMB102.

### **Study on Colonization of Strain ZY-9-13 labeled with Green Fluorescent Protein(GFP) Gene within Roots of tobacco and its Biocontrol Effect**

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Strain ZY-9-13 was isolated from fresh leaves of tobacco, which had considerably antagonism to *Phytophthora parasitica* var. *nicotianae*. The stability of the control effect mainly depends on whether antagonistic microbes could effectively colonize in plant. The purpose of the study was to explore the colonization of strain ZY-9-13 in roots of tobacco and control effect to tobacco black shank. The pattern of colonization of the tobacco was observed with fluorescent confocal microscopy and control effect was calculated after the tobacco seedling being inoculated the GFP tagged strain on tobacco black shank. The results showed that there was no obvious difference between original strain ZY-9-13 and tagged strain in antibacterial activities testing. Fluorescence microscope observation indicated that the strain ZY-9-13 propagated in the zone of root cap, elongation, maturation, and the junctions of primary roots and lateral roots. The quantities of the stain in different parts were root cap > elongation > maturation > the junctions. The pot experiment data showed that application of ZY-9-13 significantly reduced the disease incidence of black shank on tobacco. Five doses of the strain ZY-9-13 ( $1 \times 10^6$  cfu g<sup>-1</sup>,  $1 \times 10^7$  cfu g<sup>-1</sup>,  $1 \times 10^8$  cfu g<sup>-1</sup>,  $1 \times 10^9$  cfu g<sup>-1</sup>,  $1 \times 10^{10}$  cfu g<sup>-1</sup>) showed different control effect on tobacco black shank, which was 42.43%, 54.70%, 68.80%, 73.03% and 77.60% respectively. Results provided scientific basis for the strain ZY-9-13 on controlling tobacco black shank.

### **Developing a quantitative polymerase chain reaction protocol to quantitate root colonization by *Bacillus amyloliquefaciens* and *Bacillus firmus***

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*Bacillus amyloliquefaciens* QST713 and *B. firmus* I-1582 are 2 key bacterial strains which are active ingredients in commercially-available biological products that colonize plant roots promoting plant growth and offering protection against pathogens. This study was carried out to develop a qPCR protocol to understand the dynamics of root colonization by these strains. Primers and TaqMan probes were designed based on genome comparisons of the 2 strains with other publicly available bacterial genomes. The specificity of the primers was tested *in vitro* and in growth chambers focusing on corn rhizosphere. An optimized qPCR protocol was developed to quantify the number of bacteria in batch cultures and in root samples using standard curves. Corn seeds were treated with  $10^3$ ,  $10^5$  and  $10^7$  spores of the 2 strains separately and planted in sterile sand. Whole root samples were taken at 2 and 3 weeks after germination and bacterial populations were assessed. QST713 and I-1582 primers did not amplify DNA extracted from untreated corn grown soil confirming that the primers are specific. The results also show that as low as  $3.5 \times 10^3$  bacteria cells associated with 1g of corn roots can be detected using the current qPCR protocol. Furthermore, the number of bacteria cells associated with corn roots was quantified up to 3 weeks after germination. Our results show that the developed qPCR protocol can be used to understand dynamics of root colonization by plant growth promoting bacteria.

### **Effects of seed coating “inert ingredients” in the pathogenicity of *Fusarium graminearum* in soybean**

K. Navarro (1)

(1) The Ohio State University, U.S.A.

*Fusarium graminearum*, known to infect wheat, barley, corn, and other cereal crops has also been reported to cause seedling rot, root rot and pre- post emergence, damping off of soybean. Application of fungicides and other bioactive molecules are often combined with an “inert” ingredient such as sugar polymer dextrans as a seed coat to control seedling pathogens. Our study aims to investigate the effects of maltodextrin seed coating on the virulence of *F. graminearum* in soybean. Five soybean cultivars with different levels of resistance were coated with maltodextrin and inoculated with a microconidia suspension in a roll-towel assay. Our study demonstrates that maltodextrin coated soybean seeds had increase susceptibility to *F. graminearum*. Treated seedlings grew less vigorously in soil than non-treated seedlings. To address if fungal growth rates increased in the presence of maltodextrin, water agar plates amended with maltodextrin were prepared. Fungi grew faster in the presence of maltodextrin and exhibited a change in color as compared to non-amended water agar plates. Further experiments testing to address whether maltodextrin and other related sugar polymers used in seed coatings can affect fungal growth and toxin production are underway. Our study provides information about the potential for unintended adverse effects of specific “inert” ingredient in the formulation of seed coatings.

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#### **Predicting the geographic establishment of introduced mycoparasites**

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The mycoparasites, *Coniothyrium minitans* and *Trichoderma viride*, of the plant pathogens, *Sclerotinia sclerotiorum* and *Sclerotinia minor*, offer a model system for the development of an approach for predicting their geographic distribution based on the physiological responses to the environmental variables of temperature and soil moisture. A biome analysis was performed to validate presence-absence data followed by a species response to environmental parameters using CLIMEX, a climate modeling and mapping software. Biome analysis identified the % of biome types capable of supporting *Coniothyrium minitans* (21.4%), *Trichoderma viride* (14.3%), *Sclerotinia minor* (28.6%) and *Sclerotinia sclerotiorum* (21.4%). Geographic distribution of *Sclerotinia* species was based on carpogenic germination of sclerotia in response to temperature and soil moisture parameters. Mycoparasite geographic distribution was determined by conidia germination with respect to the parameters. CLIMEX model analysis reports the US locations that are very favorable for establishment for *Sclerotinia sclerotiorum* (47%), *Sclerotinia minor* (27%), *Coniothyrium minitans* (53%) and *Trichoderma viride* (20%). Areas deemed very favorable for establishment of *Coniothyrium minitans* (53%) were larger than for *Trichoderma viride* (20%). Only 3% of the areas were classified as unsuitable for *Coniothyrium minitans* as compared to 17% for *Trichoderma viride*.

#### **Differential Colonization of Tomato Cultivars by the Biocontrol Agent *Beauveria bassiana***

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Entomopathogenic *Beauveria bassiana* (Bb1198) is endophytic in tomato. Biocontrol of disease with Bb1198 is correlated with endophytic colonization. The aim of this study was to determine if colonization by Bb1198 is affected by tomato cultivar. Colonization of ‘Rutgers’ and ‘Mountain Spring’ by Bb1198 was tested in a gnotobiotic assay. Seed were treated with Bb1198 in 1% methylcellulose at  $1.6 \times 10^6$  viable CFU/seed. Control seeds received methylcellulose. Replicated tests were factorials, in a CRD. Seed germination, Bb1198 colonization, and plant growth were determined 17-21 days after treatment. Bb1198 was recovered from sections of root, stem, and leaf plated on semi-selective medium. Data were analyzed with mixed models ANOVA, and F-LSD ( $P < 0.05$ ). Germination was higher for ‘Mountain Spring’ seed treated with Bb1198 than control seed (94 vs 65%), germination of ‘Rutgers’ treated and control seed did not differ. Bb11-98 was not recovered from plants from control seed. For treated seed, colonization of ‘Rutgers’ was greater than ‘Mountain Spring’ (64 vs 29%). For both cultivars from treated seed, colonization of leaf (50%) and stem sections (67%) was greater than roots (23%). Leaf number was greatest in ‘Rutgers’ from seed treated with Bb1198, followed by control plants for both cultivars, and least in ‘Mountain Spring’ treated with Bb1198. Understanding factors that affect colonization, i.e., cultivar, could increase consistency of biocontrol by *B. bassiana*.

#### **Effect of seedborne *Xanthomonas translucens* on wheat seed germination**

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Bacterial leaf streak (BLS) has become an important disease on wheat production in Uruguay. However, very little is known about the epidemiology of this diseases. Seedborne pathogens may play an important role as primary source of inoculum but can reduce seed germination as well. The aim of this study was to investigate the effect of *X. translucens* on wheat seed germination. Seeds were collected from nine different commercial plots harvested in 2013, including different cultivars. Seeds lots were taken to the laboratory to test four seed treatments: i) check control (without treatment), ii) disinfectant (didecylidimethyl-ammonium chloride), iii) fungicide (iprodione + TMTD + carbendazim), vi) disinfectant + fungicide. Bacterial density was estimated by seed washing and dilution plating technique on Wilbrink’s agar. Seed germination was performed on paper towel. Significant differences on bacterial density were found among seed lots. Seed treatment significantly reduced the number of cfu.g<sup>-1</sup> of seed, being disinfectant the most effective reducing bacterial population. However only the fungicide showed a significant effect on seed germination, indicating that germination was not affected by the bacterial population present at the seed surface. Based on this preliminary result, seedborne *X. translucens* might play an important role as source of inoculum but did not affect germination.

#### **Characterization and Application of Oil-In-Water Nanoemulsion for Controlling Citrus Huanglongbing**

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*Candidatus Liberibacter asiaticus* (Las), which can cause destructive citrus huanglongbing (HLB), resides in citrus phloem. Based on various physiochemical characteristics of oils, surfactants, and organic solvents, oil-in-water (O/W) nanoemulsion was optimized to prepare the antimicrobial nano-formulation. The nanoemulsion was produced using a spontaneous emulsification method for the efficient delivery of antimicrobials into citrus phloem by bark application. The nanoemulsion prepared from cremophor<sup>®</sup> EL (viscous oil), acetone (water miscibility organic solvent), and Span 80/Tween 80 (surfactant) exhibited a small droplet size ( $17.33 \pm 0.52$  nm). It also had an improved absorption rate, including 2-day peak concentration ( $t_{max}$ ),  $71.86 \pm 35.38$  ng/g maximum concentration ( $C_{max}$ ), and  $267.25\% \pm 44.1\%$  relative bioavailability (RBA) of ampicillin in HLB-affected citrus compared to the Amp solution alone ( $t_{max} = 6$  days,  $C_{max} = 56.44 \pm 32.59$  ng/g and RBA = 100%). The same nanoemulsion was used to deliver five antimicrobials to control citrus HLB by bark application. We found that droplet size of antimicrobial formulation prepared in nanoemulsion was significantly reduced. And the nanoemulsion also enhanced the efficacy against Las of validoxylamine A, combination of actidione and validoxylamine A, and sulfadimethoxine sodium against Las. Therefore, this study provides an efficient self-nanoemulsifying delivery system for controlling citrus HLB.

#### **Effects of Penicillin Injection for Citrus HLB Control on Culturable Bacteria in Petioles and Rhizosphere, and Penicillin Resistance**

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Trunk injection with penicillin has been tested to control citrus huanglongbing (HLB), but environmental safety must be assured before approval of penicillin injection in groves. We investigated potential effects of penicillin injection on rhizospheric and endophytic bacterial populations using

grapefruit trees in field and greenhouse experiments. Trees were injected with penicillin G, using application rates of 0, 1000, or 6000 µg/ml. Bacteria were isolated on a low carbon medium from roots plus rhizosphere and surface-sterilized petioles at various times after penicillin injection. Selected isolates were tested for penicillin resistance (20 µg/ml) and glyphosate resistance (7000 µg/ml), because glyphosate is widely used and cross-resistance against antibiotics had been documented. Bacterial populations in petioles and root-rhizospheres initially increased after penicillin injections, possibly due to release of nutrients from dead bacteria, and then returned to control levels after one week. Penicillin resistance was common in penicillin-injected and control trees (30 to 94%). A few isolates (0 to 2%) were only resistant to glyphosate, while more isolates (29 to 30%) were only resistant to penicillin. Most isolates (54 to 71%) were resistant to both glyphosate and penicillin. Considering the widespread penicillin resistance in control soil, potential side effects of penicillin injection in citrus are small.

#### **Evaluation of a novel antimicrobial compound to control growth and biofilm formation in vitro of citrus bacterial pathogens**

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Zinkicide™ is a novel plant nutrient-based antimicrobial formulation developed for treating citrus bacterial pathogens including ‘*Candidatus Liberibacter asiaticus*’ (Las) and *Xanthomonas citri* subsp. *citri* (Xac). As per the design, Zinkicide™ is expected to enter phloem tissues of citrus plants when sprayed onto leaves for control of phloem-limited pathogens such as Las. This study investigated the effects of Zinkicide™ in vitro on growth and biofilm formation of Las surrogate *Liberibacter crescens* and Xac in batch cultures and under flow conditions using microfluidic chambers. Xac was grown in 96-well plates in Nutrient Broth and treated with serial dilutions of Zinkicide™ to test inhibition of growth and biofilm formation. Xac was also introduced to microfluidic chambers under constant media flow, and inhibitory action of Zinkicide™ on biofilm formation was recorded using time-lapse video imaging microscopy. The Zinkicide™ formulation had better antimicrobial properties compared to similar formulations with the same active ingredients, and the minimum inhibitory concentration of Zinkicide™ for Xac was calculated at 10ppm. Time-lapse video imaging showed that untreated Xac grown in microfluidic chambers formed biofilm whereas Xac treated with Zinkicide™ did not form any biofilm. In vitro results confirmed that Zinkicide™ is effective against citrus bacterial pathogens and inhibits bacterial growth and biofilm formation under flow conditions.

#### **Progress towards a novel chemical control for common scab disease of potato**

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Common scab is a globally important potato disease caused by pathogenic *Streptomyces* spp. with no one effective control strategy. Essential for disease induction is the production by the pathogen of the toxin, thaxtomin A. This study sought to develop disease control options targeting the interaction of thaxtomin A with the host, using novel chemical applications. Trials identified two compounds, 2,4-dichlorophenoxyacetic acid (2,4-D) and 2,5-dibromobenzoic acid (2,5-DBB) that when applied to the foliage of newly emerged plants prior to tuber initiation, could significantly reduce common scab tuber disease incidence and severity by over 85%, compared to the untreated control. Seed tuber treatments with 2,4-D prior to planting were also effective at reducing disease although the level of control was not as great as from foliar treatments. Disease resistance was correlated with decreased sensitivity of the tuber material from treated plants to thaxtomin A. High application rates (>200mg/L) of 2,4-D were associated with negative traits including twisted foliage, tuber deformation and yield loss. Further trials demonstrated that greatly reduced 2,4-D application rates down to 1.7-25mg/L retained effective disease control but avoided detrimental phytotoxicity effects. Field trials within Australia, China and USA testing foliar and seed treatments are on-going, with initial results indicating useful field efficacy of these chemical treatments for disease control.

#### **Antibacterial potential of Magnesium oxide nanomaterial against *Xanthomonas perforans* causing bacterial spot of tomato**

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Bacterial spot caused by *Xanthomonas perforans* can cause high disease severity on tomatoes in Florida. Due to the presence of copper (Cu)-tolerant *X. perforans* strains, copper-bactericides are of limited use; thus, there is a critical need for finding effective alternatives. In this study the antibacterial activity of magnesium oxide (MgO) and other metal oxide nanomaterials were evaluated against Cu-tolerant and sensitive *X. perforans* strains. In vitro assays demonstrated that MgO had high antibacterial ability compared to the equivalent concentration of a Cu-based bactericide against both strains. A greenhouse experiment demonstrated that disease severity was significantly reduced by MgO at 200 ppm compared to copper-mancozeb, the grower standard and the untreated control ( $p = 0.05$ ). A field experiment demonstrated that the same treatment significantly reduced disease severity compared to the untreated control ( $p = 0.05$ ). There was no significant difference in the yield between the treatments. Inductively Coupled Plasma Mass Spectrometry analysis of the fruits showed that MgO treatments did not lead to significant accumulation of Mg, Cu, Ca, K, Mn, P and S compared to the untreated. However, copper-mancozeb treatment led to significant accumulation of Cu and P compared to the untreated control ( $p = 0.05$ ). This study shows the antibacterial potential of MgO nanomaterials against *X. perforans* and its potential use against bacterial spot of tomato.

#### **Adepidyn™: A new fungicide active ingredient for control of foliar diseases**

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Adepidyn™ fungicide is a new active ingredient in the carboxamide chemical class (FRAC group 7) from Syngenta that provides excellent, long lasting control of powdery mildews, leaf spots, and other fungal pathogens on vegetables as well as row crops. The first wave crops to be registered include fruiting and leafy vegetables, cereals, peanut, grapes, potatoes, soybeans, and corn. Some of the key strengths of ADEPIDYN™ include early and late leaf spot on peanut, *Alternaria* spp., and powdery mildews. Field trials have shown the unparalleled residual control of ADEPIDYN™ fungicide, at low rates in comparison to commercial standards, providing more than three to four weeks control of *A. solani* on potato, *Cercospora* spp. on peanut, and powdery mildew on cucurbits. The high intrinsic activity and long-lasting duration of control of ADEPIDYN™ fungicide on these diseases, in rotation and in mixtures with other fungicides, will provide growers another tool for effectively managing leaf spot and powdery mildew diseases.

#### **Adepidyn™: A new fungicide active ingredient for control of Fusarium head blight on wheat**

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Adepidyn™ Fungicide is a new active ingredient in the carboxamide chemical class (Frac group 7) from Syngenta that provides broad spectrum disease control across many crops, including cereals. In addition to strong performance on various cereal leaf spots and powdery mildew, Adepidyn™ has also demonstrated efficacy on Fusarium head blight (FHB), a disease that is difficult to control and with limited management solutions. Adepidyn™ represents the only carboxamide that demonstrates good activity for control of FHB, offering growers a new tool for Fusarium control as well as providing an expanded window for application. In field trials, Adepidyn™ applied solo between BBCH 55 and BBCH 65 (Feekes 10.3 -10.52) at rates between 150 and 200 gai/ha, or at 125 to 150 gai/ha in mixture with propiconazole, provided superior efficacy to the current triazole solutions. Adepidyn™ provides excellent control of FHB and is a new mode of action for effective management for FHB in cereals.

### Quantification of the mortality of conidia of *Botrytis cinerea* and *Penicillium digitatum* in peracetic acid

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Peracetic acid (PAA) is common sanitizer, but information about its fungal toxicity is sparse. PAA toxicity was assessed to conidia of *Botrytis cinerea* and *Penicillium digitatum*, causes of gray mold and citrus green mold, respectively. They were immersed in PAA, entrapped on a glass fiber filter, rinsed with dH<sub>2</sub>O, and placed on PDA to observe germination, then the time (LT<sub>95</sub>) or concentration (LD<sub>95</sub>) when 95% mortality occurred were estimated by probit analysis. Two 100 mg/l (= ppm) solutions were used, one from 4.9% PAA + 26.5% H<sub>2</sub>O<sub>2</sub> and the other from 15.0% PAA + 22.0% H<sub>2</sub>O<sub>2</sub>. Mortality was similar in both therefore due to PAA with little or no H<sub>2</sub>O<sub>2</sub> contribution. ET<sub>95</sub> of *B. cinerea* and *P. digitatum* conidia in 100 mg/l PAA was 5.4 and 2.4 minutes, respectively. ET<sub>95</sub> of *B. cinerea* and *P. digitatum* conidia in 500 mg/l PAA was 17.2 and 9.8 seconds, respectively. LD<sub>95</sub> of *B. cinerea* and *P. digitatum* conidia during a 24-hour PAA exposure was 24.7 and 3.3 mg/l, respectively. LD<sub>95</sub> of *P. digitatum* conidia during 10 or 30-minute PAA exposure was 50 and 41 mg/l, respectively. Although PAA resistance was markedly higher in *B. cinerea* than *P. digitatum*, label rates of PAA are adequate to control conidia of both pathogens. PAA can be mixed with fungicides, label rates are relatively high (650 mg/l preharvest and 85-100 mg/l postharvest), is USDA Organic Program organic rules, and its disinfection by-products are of minimal concern.

### Effect of fungicide applications on *Monilinia fructicola* population diversity and transposon movement

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In this study we investigated whether previously reported, fungicide-induced mutagenesis observed *in vitro* in *M. fructicola* can also accelerate genetic changes in field populations. Azoxystrobin and propiconazole were applied at half label rate to nectarine trees for two consecutive years in weekly intervals for three months between bloom and harvest. Single spore isolates were obtained from blighted blossoms, corresponding cankers, and from fruit to investigate phenotypic and genotypic changes. In both years, isolate populations collected from fungicide-treated fruit and from untreated control fruit were not statistically different in haploid gene diversity ( $p > 0.45$  for 2013), ( $p > 0.46$  for 2014), allele number ( $p = 0.88$  for 2013), ( $p = 0.41$  for 2014), and effective allele number ( $p > 0.88$  for 2013), ( $p > 0.81$  for 2014). Isolates from blossoms and corresponding cankers of fungicide treatments had no changes in simple sequence repeat size or evidence for induced transposon translocation. No emergence of reduced sensitivity to azoxystrobin, propiconazole, iprodione, and cyprodinil that would indirectly indicate increased genetic diversity was detected. Our results indicate that fungicide-induced, genetic changes may not occur as readily in field populations as they do in populations exposed continuously to sublethal doses *in vitro*.

### Citrus Canker as a bioassay for systemic bactericidal activity of Zinc nanoparticles

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Huanglongbing (HLB) is caused by the phloem-limited bacterium, *Candidatus Liberibacter asiaticus* (Las), transmitted by the Asian citrus psyllid. Since August 2005, when HLB was first discovered in South Florida, citrus production has declined by more than 60%. HLB symptoms include vein yellowing, blotchy mottle, zinc deficiency, tree decline and fruit drop. Like most systemic diseases, HLB is difficult to control because foliar applied materials cannot enter the vascular tissue due to the leaf cuticle and selective permeability of cell membranes. Zinkicide™ is a plant nutrient-based nanoparticle with bactericidal activity that is designed to be translocated systemically. Prior to HLB, citrus canker was the most serious disease threat to Florida citrus. The causal agent, *Xanthomonas citri* subsp. *citri* (*Xcc*), incites leaf and fruit lesions within 2-3 weeks after infection. The goal was to investigate systemic movement of Zinkicide™ via soil drench using canker as a model for bactericidal activity because of the short evaluation time compared to HLB. In replicate greenhouse trials, soil drench and spray applications of Zinkicide™ were applied prior to leaf infiltration with 10<sup>4</sup> cfu ml<sup>-1</sup> of *Xcc*. Two weeks post inoculation lesions per leaf were counted. Zinkicide™ spray and drench applications significantly reduced lesion number. Reduction of lesions by the drench treatment indicates that systemic activity of Zinkicide™ will be useful for further evaluation against HLB.

### Evaluation of the Spatiotemporal Dynamics of Oxytetracycline and Its Control Effect against Citrus Huanglongbing via Trunk Injection

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Citrus Huanglongbing (HLB) or greening is a devastating bacterial disease that has destroyed millions of trees and is associated with phloem-residing '*Candidatus Liberibacter asiaticus*' (Las) in Florida. In this study, we evaluated the spatiotemporal dynamics of oxytetracycline in planta and its control effect against HLB via trunk injection. Las-infected 'Hamlin' sweet orange trees on 'Swingle' citrumelo rootstock at the early stage of decline were treated with oxytetracycline hydrochloride (OTC) using trunk injection with varying number of injection ports. Spatiotemporal distribution of OTC and dynamics of Las populations were monitored by HPLC method and qPCR assay, respectively. Uniform distribution of OTC throughout tree canopies and root systems was achieved 2 days post injection. High levels of OTC (>850 µg/kg) were maintained in leaf and root for at least 1 month and moderate OTC (>500 µg/kg) persisted for more than 9 months. Reduction of Las populations in root systems and leaves of OTC-treated trees were over 95% and 99% (i.e. 1.76 and 2.19 log reduction) between 2 and 28 DPI. Conditions of trees receiving OTC treatment were improved, fruit yield was increased, and juice acidity was lowered than water-injected control even though their differences were not statistically significant during the test period. Our study demonstrated that trunk injection of OTC could be used as an effective measure for integrated management of citrus HLB.

### Kasugamycin mixtures with copper, mancozeb, or a thiadiazole improve bactericidal inhibition and efficacy of treatments against walnut blight

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Resistance to copper is common in *Xanthomonas arboricola* pv. *juglandis* (*Xaj*) populations in California walnut orchards making disease management difficult when applying copper alone. The use of mancozeb in mixtures with copper led to improved disease control. The risk of resistance development to both compounds, however, is high because this has occurred with other *Xanthomonas* spp. This stresses the need for new bactericides. Kasugamycin is an effective antibiotic against several bacterial pathogens (e.g., *Erwinia* and *Pseudomonas* spp.) but strains of *Xaj* often exhibit higher minimal inhibitory concentrations (MICs) than those of other species. Our goal was to improve the activity of kasugamycin by mixing with copper, mancozeb, or a thiadiazole. In *in vitro* spiral gradient endpoint and dilution plate testing with copper-resistant strains of *Xaj*, the average MIC for kasugamycin was 27.5 mg/L. When kasugamycin was mixed with copper, mancozeb, or a thiadiazole, average MIC values were reduced to 11.2, 6.1, or 12.8 mg/L, respectively. In field trials, treatments of kasugamycin mixed with either copper, mancozeb, or a thiadiazole reduced natural disease incidence by 84.2, 81.7, or 87.8%, respectively, whereas kasugamycin alone lowered blight by 50.3%, indicating the importance of using this antibiotic exclusively in mixed treatments for increased performance. Kasugamycin is currently under federal and California regulatory review for full registration on walnut.

### Evaluation of conventional and biological pesticides for managing tomato bacterial spot during transplant production

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Bacterial spot caused by *Xanthomonas perforans*, is a ubiquitous disease of tomato in both field and transplant operations in Florida. The high prevalence of copper-tolerant bacterial strains has compromised the efficacy of traditional copper compounds. Fifteen treatments consisting of various chemical and biological pesticides alone or in programs, including a non-treated control and a copper hydroxide standard, were evaluated for their ability to control bacterial spot on tomato seedlings. Treatments were foliar applied to tomato seedlings grown in 141 cell Speedling trays maintained under conditions similar to a commercial transplant operation. Each treatment was tested at least twice in four, 3-week experiments. Disease severity was evaluated on the third week. Stand-alone applications of Actigard, Cueva, Quintec, Innovotech A, and Innovotech B significantly reduced disease compared to the inoculated controls. Programs that included Actigard + Tanos + Agriphage and Tanos + K-Phite + Cueva also significantly reduced disease compared to the control. The antibiotics, oxytetracycline and streptomycin, and Agriphage were ineffective at reducing disease severity under high disease pressure. Severe phytotoxicity was associated with Quintec at rates greater than 1 fl. oz. The copper formulation in Cueva performed better than the copper hydroxide standard. Overall, Actigard provided significant disease reduction as a stand-alone or in a program with other compounds.

#### **Nanoparticle encapsulation of seed treatment active ingredients for protection of maize seeds against *Fusarium graminearum***

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Fungicide seed treatments have long been a viable and effective strategy for protecting against seedborne and soilborne pathogens. However, losses to seeding diseases are still an important issue and there is a need for improved seed treatment efficacy. Amphiphilic polyanhydride nanoparticles have been used to enhance efficacy for several active ingredients in animal systems and have potential for agricultural use. Nanoparticle encapsulation has the potential to reduce active ingredient dosage and to provide sustained release. In order to assess these potential benefits, we developed a method to load polyanhydride nanoparticles with fungicide active ingredients to protect maize seeds against seedling blight caused by *Fusarium graminearum*. The objectives of this study were to determine the efficacy of a reduced rate of nanoparticle-encapsulated thiabendazole for suppression of seedling blight on hybrid maize seeds. Loaded nanoformulations were applied at varying rates with a planting polymer. These nanoparticle treatments were compared to a non-encapsulated thiabendazole treatment and a control. Seeds were treated and placed in a rolled towel assay for one week, to evaluate the effects on root length, shoot length, plant weight, and disease severity. Preliminary results indicate that the nanoformulated thiabendazole had significantly higher plant weight ( $p < 0.0001$ ) and root length ( $p < 0.0001$ ) than traditional thiabendazole seed treatments and inoculated controls.

#### **Evaluation of host resistance and chemical control to manage white mold of soybean in Ohio**

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White mold of soybean is caused by the fungus *Sclerotinia sclerotiorum*. In Ohio, localized outbreaks of the disease have occurred in each of the past seven years. Chemical control has had inconsistent results for disease management, especially when applied to cultivars with moderate levels of resistance. Therefore, in order to improve disease management recommendations, the objective of this study was to evaluate the efficacy of chemical control on soybean cultivars with different levels of resistance. The experiment was carried out in two fields in 2014 and five fields in 2015, all with a long history of the disease. Ten chemical treatments (untreated check, four fungicides applied at R1 or R1+R2 growth stages and one herbicide applied at R1), and two to four soybean cultivars were arranged in a split strip randomized block design with eight replications. Two fields had white mold in the untreated plots. There was significant interaction between chemical treatments and cultivars ( $P < 0.05$ ). Disease severity index (DSI) in the trials ranged from 2 to 41 in 2014 and from 14 to 44 in 2015. Applications of Endura fungicide and Phoenix herbicide significantly reduced DSI compared to the untreated control in both years. The cultivars with the highest disease resistance ratings had the lowest DSI and the highest yields. Thus, based on our results, growers should focus on soybean cultivars with the highest resistance ratings to manage white mold of soybean.

#### **Managing QoI-resistant *Cercospora sojina* in Mississippi soybean and assessing the physiological impacts of foliar fungicide phytotoxicity**

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Following the widespread detection of quinone outside inhibitor (QoI) resistant frogeye leaf spot (FLS) (*Cercospora sojina* Hara) in Mississippi during 2013 and 2014, effective management of FLS in susceptible cultivars requires the use of fungicide products that contain alternative, non-QoI, modes of action (MOA). One drawback to applying alternative MOAs, such as demethylation inhibitors (DMI), can be phytotoxicity on treated soybean leaves. Field trials were conducted in 2015 to assess fungicide efficacy on QoI-resistant FLS severity and the resulting phytotoxicity on several soybean cultivars with differing FLS reactions. The fungicides chosen have previously been observed to produce moderate to severe phytotoxicity. Six different fungicide products, comprised of single or multiple MOAs, were applied at R2, R3, and R4 along with a non-treated. FLS and phytotoxicity were visually assessed pre- and several times post-application. Plant height, number of pods and nodes were recorded to determine physiological impacts. Analyzed data revealed that certain MOAs significantly reduced FLS severity compared to the non-treated. In addition, prothioconazole resulted in significantly greater phytotoxicity than the non-treated, a difference of 29.4%. However, yield was not significantly reduced as a result of fungicide phytotoxicity. In addition, plant heights and number of nodes per plant were not significantly different between fungicide treatments and the non-treated.

#### **Management of *Fusarium* Head Blight and DON in Alabama**

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*Fusarium* head blight of wheat (FHB), caused by *Fusarium graminearum*, was severe on winter wheat in southern Alabama in the spring of 2015. A fungicide trial with four replications, planted to 'AGS 2060', allowed evaluations of demethylation inhibitor (DMI) and DMI plus quinone outside inhibitor (QoI) fungicides for managing FHB and the mycotoxin, deoxynivalenol (DON). Fungicides were applied at Feekes stage 10.5 (inflorescence complete). FHB intensity across plots was rated on a scale of 0 to 9 on 27 Apr, where 0=no disease and 9=severe disease. Plots were machine-harvested 5 Jun and kernel samples assayed for DON content. FHB in non-treated plots was rated at 7.2, which was significantly greater than FHB intensity in plots treated with propiconazole (Prop), tebuconazole (Tebu) plus trifloxystrobin (Trif), or Tebu plus prothioconazole (Proth), while Trif+Proth and metconazole (Metc) plus pyraclostrobin (Pyr) had intermediate FHB levels. DON concentration, however, was  $> 5$  ppm in grain samples from Trif+Proth- and Metc+Pyr-treated plots; only grain from Tebu+Proth-treated plots had lower DON concentrations, at 3.1 ppm, than from non-treated plots (4.6 ppm). Yield and test weight were low due to heavy disease pressure and lodging, and did not differ due to fungicide. These results confirm that a QoI fungicide, even when applied in combination with a DMI, can increase DON concentration under severe FHB pressure in wheat.

#### **Effect of leaf spot pathogens and fungicide application on Bacterial leaf streak development in wheat under field conditions in South Dakota**

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Bacterial leaf streak (BLS), caused by *Xanthomonas compastris* (*Xc*) pv. *Undulosa*, is an important disease of wheat in South Dakota. The objective of this study was to see the effect of fungal leaf spot pathogen "*Pyrenophora tritici-repentis* (*Ptr*)" (tan spot) and fungicide application on BLS development. To obtain our objectives, two wheat cultivars Briggs (BLS susceptible) and Select (moderately resistant) were planted in a randomized complete block design with three replications at SDSU Volga Agricultural Experimental Station, Volga, SD in 2014. Three plots of each cultivar was



inoculated with; Xc cell suspension alone (T1), Ptr spores suspension and then with Xc (T2), Ptr, Xc and then applied fungicide “Prosaro” (T3) and Ptr alone (T4), and non-inoculated and untreated (T5). BLS disease data was recorded 12 days post-treatments. BLS disease severity was observed higher in T2 (50.5%) as compared to T1 (Briggs =23.33%) and T3 (41.83%) in both cultivars though the severity level was higher in Briggs as compared to Select. Our results indicate that Ptr infection may help in enhancing bacterial penetration. Also, fungicide application stops tan spot development and that may help in increasing BLS development.

#### **Control of apple rots caused by *Phacidiopycnis washingtonensis*, *Phacidium lacerum* y *Diplodia seriata* in postharvest in Chile**

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The apple rots caused by fungal species in pre-harvest and postharvest are an important problem for Chilean apple industry. Recently, we detected to *Phacidiopycnis (Pha.) washingtonensis*, *Phacidium (P.) lacerum* and *Diplodia (D.) seriata* associated with apple rot in pre-harvest and postharvest in Maule Region, Chile. The objective this study were determine i) to sensitivity in vitro to fludioxonil (phenyl pyrrole), pyrimethanil (aniline-pyrimidine) tebuconazole (DMI) and thiabendazole (benzimidazole), and ii) to reduce infection with fungicide applied prior to storage on apple cv. Cripps Pink. Apple fruits were protected with different fungicides and methods (fogging and drench), and after 24 h inoculated with each pathogen. All fruit were stored at 0°C for 60 d. The results indicate that the isolates of *Pha. washingtonensis*, *P. lacerum* and *D. seriata* were sensitive to all fungicides, obtaining different EC<sub>50</sub> values. Fludioxonil, pyrimethanil, and thiabendazole by thermo-fogging reduced significantly to infection during cold storage obtaining an efficacy of 75% to 96%. Fludioxonil and thiabendazole applied by drench reached between 84% and 100% of effectivity. Finally, fungicide applied prior to storage by fogging or drench can be an effective control of apple rots during cold storage in Chile.

#### **Reducing initial infections of apple scab and black rot with salicylic acid and Actigard applied as trunk injections or sprays**

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Apple scab [*Venturia inaequalis*] and black rot [*Botryosphaeria obtusa*] are two fungal diseases that affect apple leaves early in the growing season and also make fruit unmarketable at harvest. Salicylic acid has been found to induce plant defense mechanisms to reduce initial disease infections. In this study, salicylic acid and Actigard were applied as trunk injections and spray treatments to measure their effects on the development of apple scab and black rot on established apple trees (‘Honeycrisp’ and ‘Cortland’) in the field during 2014 and 2015. Treatments were applied at the tight cluster stage of bud development and again near bloom. Incidence of leaf infections was assessed *in planta* 7, 14, and 21 days after the second treatment and disease incidence on fruit was assessed at harvest. For injection treatments, the branch directly above the injection site was assessed. In 2014, salicylic acid and Actigard applied as trunk injection or spray treatments resulted in lowest black rot and apple scab incidences on leaves and fruit compared to the control treatment. In 2015, salicylic acid and Actigard again showed a lower incidence of apple scab in the early stages of leaf infections only. No reduction in fruit infection was observed in 2015. Although salicylic acid and Actigard treatments showed a reduction in early leaf infections, apple trees must also be protected from secondary infections.

#### **Interruption and reduction of *Erysiphe necator* cleistothecia development utilizing fungicidal oil**

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Ascospores are the primary inoculum source of *Erysiphe necator* in most regions that grapevine is grown and overwinter within cleistothecia that are formed prior to leaf drop. Preventative fungicides are concluded at véraison (BBCH 83), and *E. necator* has ample time to colonize large portions of the leaf tissue, mate, and produce cleistothecia. Interruption of *E. necator* sexual reproduction post-véraison using a fungicidal oil may reduce overwintering inoculum. Chardonnay vines were sprayed once with 1% Organic JMS Stylet Oil (Vero Beach, FL) in 2014 and 2015. Fungicide application treatments were made when cleistothecia primordia were observed on leaves (BBCH 89), then 7, 14 and 21 days after primordia were observed. Cleistothecia development was monitored by weekly sampling of 10 leaves, approximately 1.4 m from the ground, per plot beginning at harvest (BBCH 89). Cleistothecia were enumerated along a transect on the leaf, and treatments were assessed using pairwise comparisons. Stylet oil applications significantly reduced cleistothecia in both years ( $P < 0.05$ ) with no effect of application timing in either year ( $P > 0.4$ ). Despite a reduction in the number of cleistothecia produced, cleistothecia were present in all plots prior to leaf drop, indicating that there may be limited utility of a late season curative fungicide application for disease management.

#### **Etiology and management of sour rot in grapes**

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Sour rot is a major challenge for affected grape growers and winemakers worldwide, yet basic disease etiology and management techniques are not well understood. Symptoms are characterized by fruit rot accompanied by the smell of acetic acid and presence of *Drosophila* (fruit fly) species. We’ve successfully reproduced these visual and olfactory disease symptoms and the accompanying characteristic production of ethanol and its conversion to acetic acid within affected berries, in the lab. Healthy fruit were wounded, inoculated with *Saccharomyces cerevisiae* and *Acetobacter aceti*, and exposed to *D. melanogaster* adults. Inoculation without exposure to flies significantly promoted ethanol production but not acetic acid generation, whereas concomitant exposure to flies resulted in both. In field trials conducted on cv. ‘Vignoles’ in 2013-15, both insecticide and antimicrobial treatments significantly reduced sour rot development. In 2015, untreated vines averaged 20.5% sour rot severity, and this was reduced by 73-81% on vines treated prophylactically post-véraison with weekly sprays containing a combination of the insecticide zeta-cypermethrin plus the antimicrobial potassium metabisulfite or hydrogen dioxide; severity was reduced by 49% on vines receiving only insecticide sprays. These trials further support the hypothesis that sour rot results from a complex of yeast, bacteria, and *Drosophila*, and that targeting these organisms can provide significant levels of control.

#### **Characterization of *Diaporthe* spp. *Cadophora malorum* and *Neofusicoccum parvum* causing cordon dieback in kiwi plant and their chemical control in Chile**

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Kiwifruits is the third largest exporter of fresh kiwifruit (*Actinidia deliciosa*) worldwide, with over 10,000 ha cultivated. Cordon dieback in kiwi plant has increased during the last decade in Chile. Recently, we have identified several species obtained from cordon dieback in kiwi plants. Therefore, the objectives of this work were i) to characterize by means of molecular and pathogenicity studies and ii) chemical control of *Diaporthe ambigua*, *Diaporthe australaficana*, *Cadophora malorum* and *Neofusicoccum parvum*. Pure cultures were characterized by phylogenetic analyses using three loci (ITS,  $\beta$ -tubulin and EF1- $\alpha$ ). Pathogenicity test were conducted on kiwifruit canes and trunks. In vitro sensitivity and protection of pruning wounds with benomyl, pyraclostrobin and tebuconazole was evaluated. This study reveals that *Diaporthe ambigua*, *Diaporthe australaficana*, *Cadophora malorum* and *Neofusicoccum parvum* are the major fungal species causing cordon dieback, and that the protection of pruning wounds with chemical fungicide is a good measure to prevent infection in commercial orchards of kiwi plants in Chile.

### **Contribution of protectant fungicides applied as mid-season cover sprays to management of peach brown rot at harvest**

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Protectant fungicides are routinely applied to peach trees during the period from shuck-split through early preharvest. These cover sprays are usually applied to control peach scab, rusty spot, and anthracnose. However, results from fungicide treatments in 2010, which lacked the typical preharvest applications, indicated that these mid-season sprays may also provide control of brown rot at harvest, caused by *Monilinia fructicola*. To confirm these observations and determine the mechanism of control, a field study was conducted from 2012-2015 in an experimental peach orchard. In each year, four protectant fungicides (captan, sulfur, ziram, and thiram) were applied from shuck-split through sixth cover. Captan and thiram in 2012 and captan in 2013 and 2015 significantly reduced brown rot at harvest. Estimation of fungicide levels on fruit in 2013 to 2015, using an *in vivo* bioassay based on *M. fructicola* spore germination, showed that residual activity was the primary mechanism for control. Results also showed that captan may have a higher intrinsic efficacy than the other protectants, thereby allowing extended control through the preharvest period. These findings indicate that residual activity from mid-season applications of protectant fungicides (i) can contribute significantly to the efficacy of preharvest programs and (ii) may play an important role in reducing selection for resistance among site-specific fungicides typically applied during the preharvest period.

### **Fungicide efficacy for the control of pomegranate diseases in Florida**

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A statewide survey identified six economically important pathogens of Pomegranate (*Punica granatum*) in Florida. Anthracnose fruit rot and leaf spot, Botryosphaeria shoot blight and stem canker, and Pilidiella fruit rot were commonly observed throughout the growing season resulting in more than 80% fruit loss. Field trials conducted at two different locations in Central Florida evaluated Cabrio, Switch, Scala, Penncozeb, and Luna Experience for disease control on pomegranate. Fungicides were applied beginning in Early-March when > 5% flower buds were visible and reapplied every two weeks until the end of growing season in August. Disease severity was rated based on the Horsfall-Barratt scale, and values were used to calculate area under disease progress curves. Significant differences were observed between the treatments at both locations ( $P < 0.001$ ). Cabrio, Luna Experience, and Penncozeb reduced the disease severity by 87, 84, and 67% respectively; while Switch and Scala reduced by 53 and 39% respectively compared to untreated control. Follow-up field studies are planned for 2016 to establish effective usage patterns.

### **Effectiveness of fungicide treatments following the Strawberry Advisory System for control of Botrytis fruit rots in Florida**

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Botrytis fruit rot (BFR) of strawberry is caused by *Botrytis cinerea*. Its management was traditionally carried out by weekly fungicide applications using multi-site fungicides in early season and single-site fungicides during bloom when the environment is conducive for disease development. Recently, many Florida growers have been following the Strawberry Advisory System (SAS) to improve timing and reduce the number of fungicide applications. Our objective was to determine the efficacy of different fungicides for BFR control when following the SAS spray timing alerts compared to preventive Calendar-based applications, and to implement specific fungicide recommendations on SAS. Field trials conducted during two seasons on a commercial farm in Plant City-FL showed that during the highest disease incidence periods, treatments with cyprodinil + fludioxonil or fluopyram following SAS and Calendar-based applications were the most effective in controlling BFR. Pyrimethanil and boscalid + pyraclostrobin were not effective due to resistance problems and are no longer recommended in the SAS. For SAS alerts during absence of bloom, a multi-site fungicide such as captan or thiram is recommended. Treatments following SAS provided good control of BFR with about half the number of fungicide applications compared to Calendar-based applications. However, the choice of the fungicide when weather conditions are highly favorable for disease development is the key for effective control.

### **Interaction among leaf area index, spray volumes and fungicide doses in Asian soybean rust control**

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Soybean cultivars vary in their leaf area index (LAI), which influences the distribution of the drops generated by the spray fungicides in plant canopy. The objective of this study was to investigate the interaction among the LAI, spray volumes and fungicide doses in controlling Asian soybean rust. The tests were conducted at UPF experimental field, greenhouse and growth chamber during 2014 to 2016. The dose was varied (75%, 100% and 125% of the commercial dose) and the spray volume (100, 150 and 200 L/ha) at application of fungicides on soybean plants with different LAIs. An assay was also conducted with the use of simulated rainfall at different times after the fungicide application. There were three cultivars in the experimental field: BMX Ponta (high LAI); BMX Vanguarda (medium), NS 5445 (low), and only the last one in the other trial. The applications were in V7 stages (trifloxystrobin+prothioconazole), R1 and R1+20 days (azoxystrobin+benzovindiflupir) for field trials and only in V8 stage for testing in the greenhouse. The severity of the rust, the number of spores/cm<sup>2</sup>, the LAI and the grain yield were quantified and analyzed by Anova and Skott-Knott test (0.05). The results showed that the spray volume and the fungicide dose should be appropriate according to the cultivar LAI. The closer the application when rain occurs, lower the control achieved by applying the fungicide. The cultivar LAI, spray volume and the fungicide dose influenced the disease control.

### **Relationship between fungicide dose and Asian soybean rust control**

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The diseases affect the soybean crop yield, and the main one is the Asian soybean rust (ASR) and the control of the disease is mainly based on fungicides. The objective of this study was to verify if the use of 0 to 100% of the commercial dose of fungicide affects the control of the disease. The experiment was conducted in a growth chamber at UPF, in a completely randomized design with six replications. For this, the central leaflets of soybean plants (NS 5445 cultivar) grown in a greenhouse were collected. The solutions from 0 to 100% of the dose were prepared using two fungicides, one consisting of trifloxystrobin + prothioconazole and other azoxystrobin + benzovindiflupir. The leaflets were dipped in appropriate fungicide solutions and placed in gerboxes, where humid chambers were set up. In the next day, a suspension of *Phakopsora pachyrhizi* (50,000 spores/mL) was prepared, which was sprayed on the leaflets. The gerboxes were left in the dark for 24 hours at a temperature of 23°C. After this, they were placed on benches in a growth chamber with a photoperiod of 12 hours light/dark and constant temperature of 23°C. After 22 days incubated, the number of spores/cm<sup>2</sup> was assessed. The controls without application of fungicides showed an average of 85.4 spores/cm<sup>2</sup> (T+P) and 83.5 spores/cm<sup>2</sup> (A+B). There was a reduction in the number of spores/cm<sup>2</sup> when increases the fungicide dose. The ASR control depends on the fungicide dose in the spray solution.

### **Response of Early and Late Leaf Spot Diseases on Peanut to QoI Solo Spray Programs**

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Foliar diseases including rust (*Puccinia arachidis*), early leaf spot (ELS; *Cercospora arachidicola*) and late leaf spot (LLS; *Cercosporidium personatum*) are annual problems for southeast peanut producers. Fungicides are a critical part of an integrated management program for these devastating peanut

diseases. The study objectives were to examine the efficacy of commonly used QoI fungicides for managing these diseases and to collect isolates for future pathogen population studies. Field plots of the peanut cultivar Georgia-06G were established after June 1st during 2014 and 2015 growing seasons. The fungicides azoxystrobin (Abound 2.08 SC at 18 fl oz/A) and pyraclostrobin (Headline at 9 fl oz/A) were individually applied every 12 to 17 days starting about 30 days after planting for comparison with untreated controls. Disease severity and incidence were assessed using the Florida 1-10 scale and by sampling 12 leaflets per plot. The predominant disease in 2014 was LLS and ELS in 2015. Azoxystrobin and pyraclostrobin treated plots had similar AUDPC values in 2014 and were significantly less than those of untreated plots ( $P \leq 0.05$ ). Mean AUDPC values were not significantly different in 2015. These results suggest that QoI fungicides have greater efficacy for LLS, however, they do not provide adequate control of ELS. Further *in vitro* investigations are ongoing in Florida to examine the population diversity of these pathogens and their QoI fungicide resistance profiles.

#### **Effect of fungicide timing and frequency on reducing foliar diseases and yield loss on runner and valencia type peanuts in Haiti**

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Peanut (*Arachis hypogaea* L.) yields in Haiti are often severely reduced by peanut rust (*Puccinia arachidis*) and late leaf spot (*Cercosporidium personatum*). Two trials were conducted in 2015 to evaluate the effect of 6 fungicide regimes on disease severity and yield for two market types. Both tests used a split plot design with 4 replications with variety as whole plot and fungicide as subplot. Runner (local landrace and Cv. Georgia-06G) and valencia (local landrace and Cv. N.M. Valencia A) market types were planted in northern and central Haiti, respectively. For runners, Muscle ADV (2.3 L/ha) was applied 2-6 times starting 30-60 days after planting (DAP) with 14-28 day intervals; valencias received 1-4 sprays starting 30-45 DAP with 14-28 day intervals. There were no interactions between variety and fungicide regime. At each location, disease severity was similar between varieties, but Georgia-06G and the local valencia yielded 2500 and 430 kg/ha more, respectively. For valencias, at least 2 sprays were required to reduce severity and increase yield; however, 3 or more sprays gave the highest yields. For runners, 3 and 4 sprays were required to reduce rust and leafspot, respectively, but 6 sprays had the least disease and the only statistically different yield. Compared to untreated plots, the best treatments increased yields by 68% for both market types. These results are a significant step toward developing management recommendations for growers in Haiti.

#### **Fungicides for management of *Sclerotium rolfsii* and enhanced overwintering survival of stevia**

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Stevia (*Stevia rebaudiana*) is an herbaceous perennial under evaluation as a new crop in NC. Stem rot caused by *Sclerotium rolfsii* results in wilting, rapidly followed by root and stem necrosis. Currently there are no fungicides labeled for use on stevia, which will limit expansion of commercial production. Initial fungicide efficacy trials were established in July 2014 in naturally infested soil in Kinston, NC. Treatments included azoxystrobin, flutolanil, and tebuconazole applied at 3 timings. This trial was repeated in May and July of 2015 in adjacent field space. Stand counts were taken to determine disease incidence and overwintering survival of stevia crowns. All fungicides reduced incidence of stem rot compared to the control. In 2015, azoxystrobin was correlated with enhanced overwintering survival (mean survival rate, 66%) compared to other fungicide treatments (19%) and the untreated control (16%). In 2015, a second field site was established in Rocky Mount, NC to evaluate the effect of late season applications of azoxystrobin and pyraclostrobin. Both fungicides resulted in higher root weights and greater new shoot production compared to the control in spring 2016. Final stand counts of all treatments will be taken in spring 2016 to establish overwintering survival rates. Successful overwintering of stevia crowns directly impacts the profitability of second and third year harvests and the likelihood of long term establishment of stevia in NC.

#### **Timing of fungicide applications is critical for blackleg control in canola**

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Blackleg, caused by *Leptosphaeria maculans*, is an important disease affecting rapeseed/canola (*Brassica napus*) production worldwide. The genetic resistance currently available in most commercial cultivars is ineffective against strains of blackleg that have become prevalent in recent years and because of this, growers are relying more frequently on fungicide use to manage this disease. To optimize fungicide use, greenhouse studies evaluated the efficacy of different timings of application of azoxystrobin, pyraclostrobin, and fluxapyroxad on blackleg severity. These active ingredients, present in four of the six fungicides registered to manage blackleg in North Dakota, were applied two days before and 0, 2, 4, 8, or 16 days after seedlings of cv. Westar were inoculated with an *L. maculans* spore suspension. Disease severity was evaluated 12 days after inoculation and before harvest. For each fungicide, trials were conducted separately using a randomized complete block design. Each study was replicated and conducted three times. All three fungicides were most effective when applied within eight days from inoculation and lost their efficacy when applied at a later time. This information highlights the need for early protection of seedlings as well as for the development of disease warning systems that could identify help growers optimize spraying schedules.

#### **Evaluation of fungicide efficacy and cultivar susceptibility for the management of anthracose on celery**

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Anthracose on celery, also called celery leaf curl, is caused by *Colletotricum fiorinae*. The disease was first identified in Ontario, Canada, in 2011 and has been increasing in severity. Field trials were conducted to determine if there were differences in cultivar susceptibility and to identify effective fungicides. The celery cultivars were: TZ6010, TZ0295, TZ6200, TZ6029, TZ9779, Nero, Sabroso and Plato. Cv. TZ 6200 was used for the fungicide trial. Seedlings were transplanted on 24 June into 'muck' soil (organic matter ~70%, pH 6.5) in the Holland Marsh, Ontario. Each replicate consisted of 3 rows, 55 cm apart and 5 m in length. The trials were inoculated on 18 August with a suspension of  $1 \times 10^5$  conidia ml<sup>-1</sup>, and harvested on 13 October. The fungicides evaluated were Omega (fluazinam 40.0%), Bravo (chlorothalonil 50%), and Flint (trifloxystrobin 50%). Fungicides were applied on 31 July, 13 August and 8 September, 2015. Disease incidence and marketable yield were assessed. Cultivars Plato, TZ 9779, 6200 and 0295 had high disease incidence (86, 66, 66, and 67%) compared to less susceptible cv.'s Sabroso (24%) and TZ 6010 (27%). Fungicides Flint and Bravo had low disease (34, 45%), compared to the untreated check (83%). Increased disease incidence in September was related to a decrease in the percent of marketable yield by weight ( $r^2=0.45$ ).

#### **Optimizing the systemicity of triazole formulations by enhancing leaf penetration**

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The systemicity of a fungicide plays a vital role in its performance. While the ability of a fungicide to penetrate the leaf cuticle and translocate through plant tissue is active specific, inert formulation ingredients can optimize these processes on target crops. Triazoles and strobilurins are broad spectrum fungicides with known systemicity; however, in lab testing only a small fraction of applied active ingredient was able to penetrate a synthetic membrane that mimics a leaf cuticle. Fungicide formulations that are optimized for membrane penetration may have enhanced disease control over existing formulations. Laboratory trials were conducted using a Franz cell fitted with a synthetic membrane to identify experimental formulations that may have better leaf cuticle penetration than the commercial product. Field trials were conducted in 2015 to evaluate the efficacy of experimental tebuconazole formulations on powdery mildew in acorn squash. All tebuconazole treatments were applied weekly at label rate following disease onset for a total of

four applications. While yield did not differ statistically between any of the treatments, an experimental formulation significantly reduced disease severity by 40.5% relative to the commercial product and 55.3% relative to the untreated control. From the positive field results achieved by enhancing leaf cuticle penetration, further field and lab testing to optimize performance will be completed.

#### **Season long management of potato silver scurf caused by *Helminthosporium solani* in Wisconsin**

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Silver scurf, caused by the fungus *Helminthosporium solani*, is a tuber blemish disease of increasing concern in potato production. The disease affects periderm quality and appearance, impacting marketability as well as storability. Cultural management strategies are limited and often impractical for large-scale growers, making fungicide applications an important component of integrated silver scurf management. *Helminthosporium solani* initially infects tubers in the field, and has secondary infection cycles during storage. Because inoculum can be reduced at multiple times during potato production, we conducted a full life cycle study in 2015 to evaluate the aggregated impact of fungicide selection and timing on silver scurf severity in 'Dark Red Norland' potatoes. Fungicides were applied at three times: at planting as seed or in-furrow treatments; in-field as a series of 3 foliar sprays; and post-harvest immediately before storage. Non-treated comparisons were included. The at-planting applications had the greatest impact on symptom severity, although the post-harvest treatments also contributed to disease reductions. The lowest severity resulted from the Maxim MZ (fludioxonil + mancozeb) seed treatment with the Stadium (fludioxonil + azoxystrobin + difenoconazole) post-harvest treatment. Along with cultural methods, at-plant and post-harvest fungicide treatments together were necessary for silver scurf control.

#### **Greenhouse characterization of hydrazone-copper mixtures as low copper treatments to control early and late blight on tomato**

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Copper-based fungicides remain critical tools in crop disease management programs to control a broad spectrum of fungal and bacterial plant pathogens. However, because of the high rate required to control plant pathogens, there is concern regarding their potential negative environmental impact. Certain salicylaldehyde benzoylhydrazone (SBH) compounds bind copper and show strong synergism with copper when tested for inhibition of fungal growth under *in vitro* conditions. Experiments were conducted to characterize synergism for Cu-hydrazone mixtures in controlling *Phytophthora infestans* and *Alternaria solani* on tomato under greenhouse conditions. Treatments included a SBH alone, three copper materials, and Cu-SBH mixtures. The SBH alone was weakly active against both pathogens with 26 and 33% disease control at 400 micromolar for *P. infestans* and *A. solani*, respectively. The copper materials, which included Cu(OH)<sub>2</sub> in the form of Kocide® 2000, CuSO<sub>4</sub>, and CuCl<sub>2</sub>, provided a similar level of disease control (*p* > 0.3) when applied alone. The copper-SBH mixtures at various molar ratios produced synergistic interactions in nearly all combinations and were superior to the commercial fungicide Kocide® 2000 at equivalent rates of copper.

#### **Yield loss and management of orange cane blotch of blackberry, caused by the alga *Cephaleuros virescens***

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The alga *Cephaleuros virescens* causes orange cane blotch (OCB), a serious disease of blackberries in the southeastern United States. OCB impact on blackberry growth and yield was assessed. Neither cane diameter nor fruit size were significantly impacted by severity of OCB; however, maximum fruit number per cane decreased by 80% with increasing OCB severity, thereby resulting in reduced yields. Field efficacy trials with diverse algicides, disinfectants, and fungicides were conducted over a 3-year period from 2013 to 2015 at two locations on a thornless blackberry cultivar, 'Ouachita'. Treatments, applied during the summer and fall, included ametoctradin + dimethomorph, calcium polysulfide, calcium polysulfide + surfactants, captan, chlorothalonil, copper hydroxide, copper hydroxide + hydrogen dioxide, fluazinam, fluopicolide, hydrogen dioxide, mancozeb, mancozeb + copper hydroxide, mandipropamid, mfenoxam, mfenoxam + copper hydroxide, mfenoxam + mancozeb, potassium phosphite, potassium phosphite + captan, potassium phosphite + copper hydroxide, oxathiapiprolin and diluted sodium hypochlorite. Potassium phosphite, generally active on both oomycetes and fungi, was the only chemical that provided significant suppression of the disease, with 48-80% reduced lesion coverage of canes. Management of OCB will be difficult in light of the limited number of labeled applications of potassium phosphite allowed and the potentially long infection period of the pathogen.

#### **Evaluation of Nematicides for Control of Southern Root-Knot Nematodes in Lima Bean**

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Southern root-knot nematodes (*Meloidogyne incognita*-RKN) significantly reduce lima bean (*Phaseolus lunatus*) yields. Chemical control options for RKN are limited. We evaluated the efficacy of new nematicides on RKN in lima bean experiments conducted in greenhouse (GH) and microplot (MP) settings. Treatments included Nimitz at low and high labeled rates, Luna, Movento, Luna+Movento, Vydate, Mocap and an untreated, RKN infested control. GH treatments were arranged in a factorial design, with application of nematode eggs (0, 6,000, or 30,000 eggs pot<sup>-1</sup>) crossed with nematicide treatment. MP treatments were arranged in a randomized complete block design and all plots were infested with 4.80 kg of RKN infested tomato root tissue and soil. Root galling, RKN eggs and juveniles, and aboveground plant dry masses were determined and statistically analyzed using Mixed Model ANOVA. In the GH study, Nimitz at both rates provided the greatest reduction in RKN galling compared to the controls, whereas Movento treatments were not significantly different than the controls. In the MP study, all treatments except Movento significantly reduced RKN populations and had significantly greater yields relative to controls. Vydate, Mocap, and Nimitz (high and low rate) treatments had the highest yields in the MP study. Based on these results, several new nematicides show potential for managing RKN in lima beans.

#### **Evaluation of soil conditioner OR079 for the optimization of metalaxyl in control of *Phytophthora nicotianae* in Texas citrus orchards**

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The Texas citrus industry faces a great problem with the control of foot and root rot disease caused by *Phytophthora nicotianae*. *P. nicotianae* infects feeder roots and the trunk of trees causing poor water and nutrient uptake leading to yield loss, fruit size reduction and tree decline. Treatments for *Phytophthora* diseases are limited, therefore optimization of available products is of great importance. We hypothesized that the effectiveness of metalaxyl in reducing *P. nicotianae* soil propagules can be increased when applied in combination with the soil conditioner OR079. To test our hypothesis we applied metalaxyl, OR079, and metalaxyl plus OR079 to mature grapefruit trees with *Phytophthora* infection and quantified *Phytophthora* propagules in the soil. *Phytophthora* propagule counts were assessed at 4, 8, and 12 weeks post treatment and compared to an untreated control. Results showed that all treatments had significantly reduced *Phytophthora* counts in the soil at 4 and 8 weeks post application compared to that of the untreated control, but were not statistically different to the control by 12 weeks. Moreover, OR079 did not increase metalaxyl effectiveness in reducing *Phytophthora* propagules but reduced its activity when mixed. In addition, application of OR079 and metalaxyl significantly increased citrus fruit size. These results indicate that timely treatment for *Phytophthora* infection improves root health and water uptake leading to increase citrus fruit quality.

### **Efficacy of Oxathiapiprolin toward *Phytophthora sojae* and *Phytophthora sansomeana***

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Poor crop stand establishment caused by soil-borne pathogens is very common in soybean fields in Ohio. Metalaxyl has been the most commonly used fungicide to control *Phytophthora* and *Pythium* species in soybean. However, several studies have reported an increase of insensitivity to metalaxyl in *Phytophthora* and *Pythium* populations in Ohio. Therefore, alternative fungicides should be tested for efficacy towards *Phytophthora* and *Pythium* species. The objective of this experiment was to determine the efficacy of oxathiapiprolin in reducing the growth of *Phytophthora sojae* and *Phytophthora sansomeana* in soybean. A total of 28 isolates collected from infested fields were evaluated using amended agar Petri dishes with 5 different concentrations of oxathiapiprolin. Sensitivity of each isolate was evaluated by estimating the diameter of mycelial growth on each Petri dish and comparing it to the control. Results from preliminary data show that oxathiapiprolin treatments reduced *P. sojae* and *P. sansomeana* mycelial growth and mean values varied among isolates. Eight isolates did not grow at 0.01 µg active ingredient/ml. Based on these results could be used to manage seed rot caused by *P. sojae* and *P. sansomeana*.

### **Efficacy of three soybean fungicide seed treatments against *Pythium* species in seed plate and growth chamber assays**

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Recent studies have reported a wide range of sensitivity to metalaxyl in *Pythium* species in Ohio, thus new active ingredients are important for management of seed and seedling blight. The objective of this study is to determine the efficacy of three seed treatments with a combination of active ingredients for oomycetes against a diverse population of *Pythium* species. Commercial formulations of Intego™ Suite, Cruiser Maxx® Advanced, and Acceleron® were applied to seed at labeled rates and evaluated with *Pythium* isolated from fields across Ohio in both a plate assay and a cup assay. In the cup assay, the mean root score was significantly lower in all fungicide seed treatments compared to the control, but there were no significant differences between treatments. In the seed plate assay, there was a significant difference for mean seed rot score between all treatments. Acceleron® had the lowest seed rot score towards all *Pythium* species, followed by Intego™ Suite, Cruiser Maxx® Advanced, and the non-treated check, respectively. These seed treatments may provide adequate protection against *Pythium* infection in soybean seed and seedling stages. In addition, the seed plate assay may be used to efficiently test for isolate pathogenicity and the efficacy of seed treatments.

### **Preventative fungicide applications in production and their impact on residual efficacy against impatiens downy mildew in the landscape**

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Since the emergence of impatiens downy mildew researchers have come to the conclusion that chemical control is the only effective short-term management option available. The greatest challenge is identifying cost effective management options for impatiens downy mildew in the landscape. Some of our first trials conducted early in the spring of 2012 revealed that impatiens transplanted into landscape beds incorporated with a granular form of mefenoxam remained healthy for 60 days. Subsequent experiments evaluating products for long residual (> 30 days) control have identified several additional chemistries including fluopicolide, various phosphonates and oxathiapiprolin as promising. Further studies investigated the potential for eradication control and found phosphonate containing products effective even after infection. Most recent studies have focused on fungicide rotations targeting long term residual control of impatiens downy mildew. Weekly preventative fungicide spray rotations on potted impatiens in production have included cyazofamid, dimethomorph, fluopicolide, mefenoxam, potassium phosphite and oxathiapiprolin. To date, oxathiapiprolin has provided stellar control of impatiens downy mildew when used alone and in every rotation study. Results obtained from fungicide efficacy trials conducted over the past four years indicate that some chemistries provide long enough residual control for impatiens to remain untreated in the landscape.

### **Combined Application of *Bacillus amyloliquefaciens* ZY-9-13 with Fungicides for Control of Tobacco Black Shank**

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Tobacco black shank is a devastating soil borne disease caused by *Phytophthora parasitica* var. *nicotianae*, which greatly reduces tobacco yield and quality. Fungicides, such as metalaxyl, dimethomorph and mancozeb, have been widely used to prevent and control this disease. Integrated biological and chemical control can release pressures from environment and delay emergence of fungicide resistance. *Bacillus amyloliquefaciens* ZY-9-13 has good control effect to tobacco black shank in our previous studies. In this research, we 1) assessed sensitivities of *P. nicotianae* to metalaxyl and dimethomorph, 2) mixed *B. amyloliquefaciens* ZY-9-13 and fungicide metalaxyl, dimethomorph, mancozeb in order to provide an alternative disease control option. The baseline sensitivities of *P. nicotianae* to metalaxyl and dimethomorph were described by unimodal curves, with mean EC50 values of 0.0664(±0.0282) and 0.3502(±0.0332) µg/ml, respectively, which can be used for detection of resistance isolates. Laboratory tests showed that fungicide mixture of metalaxyl, dimethomorph and mancozeb had additive effect, and the inhibition ratio of ZY-9-13 to fungicide mixture was less than 10% even at high concentration. The integrated biological and chemical control showed a synergistic action. In the field trial, disease control with the combination of *B. amyloliquefaciens* ZY-9-13 and fungicide mixture was 79.76%–86.90%, which was greater than with ZY-9-13 or fungicide alone.

### **Laboratory and field evaluation of fungicides for management of Phytophthora blight of pumpkin, caused by *Phytophthora capsici***

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Phytophthora blight, caused by *Phytophthora capsici*, is a destructive disease of cucurbits and peppers in Illinois and worldwide. Fungicide use is an important component of management strategies of Phytophthora blight of cucurbits. Studies were conducted in laboratory and field to evaluate efficacy of selected fungicides for management of Phytophthora blight of pumpkin in Illinois. In the laboratory, sensitivity of five *P. capsici* isolates from Illinois was evaluated to three registered fungicides (cyazofamid, dimethomorph, mandipropamid) for use on cucurbits and two experimental fungicides (A20941OD, A20942SC) for control of *Phytophthora* spp. Development of colony and production of sporangia and zoospores of the isolates were assessed at different concentrations from 0 to 10 µg/ml for registered fungicides and from 0 to 200 µg/ml for experimental fungicides on V8 juice agar medium in Petri plates. The isolates were highly sensitive to mandipropamid, intermediately sensitive to cyazofamid and A20941OD, and less sensitive to dimethomorph and A20942SC. In the field experiment in 2014, incidence of pumpkin fruit rot caused by *P. capsici* was 31% in control plots and 5, 10, 2, and 11% in the plots treated with cyazofamid, dimethomorph, mandipropamid, and A20942SC, respectively. In 2015, incidence of pumpkin fruit rot caused by *P. capsici* was 69% in control plots and 42, 21, and 18% in the plots sprayed with cyazofamid, dimethomorph, and mandipropamid, respectively.

### **Fungicide rotation schemes and Melcast for managing Phytophthora fruit rot of watermelon in Southeastern United States**

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Phytophthora fruit rot (*Phytophthora capsici*) limits watermelon production in most states in the Southeastern US. We identified several effective fungicides (Revus, Presidio, and Zampro) to manage Phytophthora fruit rot in our previous studies. In this study, the effectiveness of fungicide rotations

and spray applications based on Melcast for managing Phytophthora fruit rot were conducted in *P. capsici* infested fields at three locations in southeastern US (NC, SC, GA) in 2014 & 2015. The triploid seedless cv. Wonder was used at all locations with diploid cv. Mickylee as the pollinizer. Five weekly foliar application of fungicides were made at all locations. Significant fruit rot was observed in non-treated check plots in both years at all three locations (Mean=68%). All the fungicide rotation schemes significantly reduced Phytophthora fruit rot compared to non-treated check. Overall the rotation scheme of Zampro (dimethomorph+ametoctradin) alternated with Orondis (oxathiapiprolin) was very effective across three locations. Revus (mandipropamid) alternated with Presidio (fluopicolide) was similarly effective in SC. MELCAST reduced one spray application in SC in 2014 but not in NC or GA, and one spray in GA and SC in 2015 with reduction in fruit rot similar to other rotation schemes. Fungicides in different FRAC groups are currently available for use in rotations schemes to manage Phytophthora fruit rot and Melcast may also be useful for scheduling spray treatments.

#### **Characterization of Zinkicide™ in plant tissues**

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The unprecedented crop losses caused by the high incidence of huanglongbing (HLB) vectored by the Asian citrus psyllid (ACP) in Florida citrus groves calls for the rapid development of an effective product available to growers for HLB management. Zinkicide™ is a prototype plant nutrient-based nanoparticle developed to provide growers with a new and effective systemic bactericide that will translocate into plant tissues to target the phloem-limited bacterium, *Candidatus Liberibacter asiaticus* (Las), responsible for infection and damage of internal phloem tissues in the plant associated with HLB. Unlike traditional bactericidal management, we show that the new Zinkicide™ treatment allows systemic movement to reach phloem tissue and interact with Las with ongoing assessment of tree health responses in field trials. To complement green house and field trial data, we explore the fundamental properties of Zinkicide™ formulations and their interactions with bacteria and citrus plant tissues. Morphology, structure and composition of the formulations were determined using a combination of electron microscopy, UV-Vis, fluorescence and infrared spectroscopy measurements. In addition, correlations with antibacterial efficacy, phytotoxicity, particle uptake and evolution in planta were established by monitoring the fluorescence, lifetime and infrared signatures of the particles as they penetrate into the plant and interact with the phloem tissues.

#### **Monitoring Pennsylvania orchards for the presence of streptomycin resistant *Erwinia amylovora***

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Within the last 40 years, streptomycin resistant strains of *Erwinia amylovora* have emerged in apple orchards throughout the United States. To date, resistant strains of *E. amylovora* have not been identified within Pennsylvania; however, they have been found within neighboring states such as New York, raising concerns of resistant strains soon surfacing in Pennsylvania orchards. The purpose of this study was to determine if streptomycin resistant strains are present in apple orchards located throughout Pennsylvania. In 2015, isolates were collected from grower submitted fire blight samples to the Pennsylvania State University Fruit Research and Extension Center in Biglerville, Pennsylvania. Initial identification was performed using Crosse Goodman specific media and apple inoculation. *Erwinia* identification was confirmed with direct colony PCR using primers specific to plasmid Ea29. Positive isolates were screened for resistance using a disk diffusion assay (100 µg/ml and 2500 µg/ml of streptomycin sulfate respectively). Resistance was evaluated by observation of the presence or absence of a zone of inhibition. Of the samples processed to date, none have demonstrated streptomycin resistance. Screening is ongoing and any resistant isolates obtained will be further evaluated for the presence of plasmid mediated and chromosomal genes involved in resistance.

#### **Effect of kasugamycin application on bacterial resistance to kasugamycin and cross resistance with other antibiotics**

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Kasugamycin (Ks) is an alternative option to the use of streptomycin for the control of bacterial plant diseases and it has been shown to be as effective as streptomycin in controlling fire blight, caused by *Erwinia amylovora*. However, there remains a concern that Ks application in orchards will select for Ks resistance that could be linked with other resistance genes that are active against antibiotics used in human medicine. To monitor for this possibility, we assessed the effect of the use of Ks (Kasumin 2L) in orchard systems on the level of resistance to Ks and five other antibiotics (streptomycin, ampicillin, gentamicin, cefotaxime, and tetracycline). Two sets of leaf and soil samples were collected (treated and non-treated with Ks) from a total of 23 orchards (5 different tree hosts from 7 states). Samples were processed in the laboratory and dilution plated onto King's B medium with or without Ks amendment. Bacterial population sizes were determined per sample and up to 15 gram-negative colonies growing on Ks-amended medium per sample (a total of 1,038 isolates) were used in antibiotic resistance screening. Although the bacterial population sizes were larger on Ks-amended medium from soil compared to leaf samples, there were no differences in populations from Ks treated vs. non-treated sites. Similarly, there was no difference in levels of resistance to the five antibiotics tested between Ks-treated and non-treated sites.

#### **Fungicide sensitivity in the wild rice pathogen *Bipolaris oryzae***

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In recent years the occurrence of fungal brown spot, caused by *Bipolaris oryzae* has increased in cultivated wild rice (*Zizania palustris*) paddies in spite of the use of fungicides. To implement an efficient integrated disease management system, we are exploring whether field isolates have developed reduced sensitivity due to the recurrent use of strobilurin (i.e. inhibitors of the quinone outside site of the mitochondrial cytochrome *b*, QoI) and azole (i.e., C14 demethylation inhibitors, DMI) fungicides. We conducted in vitro spore germination assays to determine the EC<sub>50</sub> of two sets of isolates collected in 2008 and 2015 using 0, 0.10, 0.15, 0.25, 0.35, 0.5, 1.0, 5.0, 10.0, and 100 µg/ml of the active ingredient azoxystrobin (QoI), and 0, 0.1, 0.15, 0.25, 0.5, 1, 5, 10, 35 µg/ml propiconazole (DMI). All isolates tested were found to express the mitochondrial alternative oxidation pathway (AOX) allowing the fungus to germinate at all the doses of azoxystrobin tested. When the AOX was blocked, an average EC<sub>50</sub> value of 0.206 µg/ml was obtained for 2015 isolates and 0.158 µg/ml for isolates collected in 2008. Up to now, there is no evidence that isolates have become less sensitive to QoI fungicides. The EC<sub>50</sub> obtained for propiconazole for all strains tested until now was lower than the dose-equivalent used in the field. Molecular and in planta tests are underway to test the efficacy of the AOX pathway in spore germination.

#### **High-throughput strobilurin sensitivity testing of *R. solani* on Louisiana rice confirms reduced azoxystrobin but continued trifloxystrobin sensitivity**

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Azoxystrobin resistance in the rice sheath blight pathogen, *Rhizoctonia solani* AG 1-IA, has compromised a widely effective disease management tool for rice production in southwestern Louisiana. We previously characterized azoxystrobin, pyraclostrobin, and trifloxystrobin sensitivity of 40 *R. solani* isolates, one from each of 40 rice fields, to identify locations with azoxystrobin resistance and to assess cross-resistance. A high-throughput fungicide sensitivity assay was developed and used to characterize 122 additional isolates from these 40 fields. Serial dilutions of commercial grade fungicides were made in potato dextrose broth and added to 96-well microtiter plates. Fungal mycelial growth was measured based on optical density. Growth

curves were generated and used to determine EC<sub>50</sub> values, resulting in sensitivity distributions that agree with previous results. The distributions of azoxystrobin and pyraclostrobin EC<sub>50</sub> values were bimodal while trifloxystrobin was not. This and internal control isolates provide significant evidence of azoxystrobin resistance, reduced pyraclostrobin sensitivity, and continued sensitivity to trifloxystrobin in the sampled *R. solani* population. Growers need to prepare for the advent of azoxystrobin-resistant *R. solani* and could potentially substitute trifloxystrobin in fungicide rotations.

#### **Resistance to quinone outside inhibitor fungicides in *Cercospora sojina* in Ohio**

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Quinone outside inhibitor (QoI) fungicides are a common class of fungicides used to manage the fungus *Cercospora sojina*, the causal agent of frogeye leaf spot in soybean. Soybean leaf samples exhibiting symptoms of frogeye leaf spot were sent to the Soybean Pathology lab from across the state of Ohio during the summer of 2015. *Cercospora sojina* conidia were recovered from lesions to test their sensitivity to QoI fungicides. Potential *C. sojina* samples were confirmed by ITS sequencing then tested for the presence of the G143A mutation in the cytochrome b gene, the most commonly observed mutation in QoI-resistant fungi. QoI fungicide resistance was detected in two *C. sojina* samples in a mixed population of sensitive and resistant spores, while all other samples were sensitive to QoI fungicides. Fast and efficient identification of fungicide resistant *C. sojina* in Ohio will be useful in minimizing potential soybean yield loss as frogeye leaf spot incidence in Ohio increases in the warm, moist conditions and mild winters that favor the pathogen.

#### **Evaluating fungicide sensitivity of regional populations of *Blumeria graminis* f.sp. *tritici* in the United States**

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*Blumeria graminis* f.sp. *tritici* (Bgt), the causal agent of wheat powdery mildew, is primarily controlled by resistant wheat varieties and foliar fungicides. However, both control strategies risk being defeated by the large, rapidly adapting pathogen population. In 2013 and 2014, over 350 Bgt isolates were sampled from 27 fields in 15 states. The fungicide sensitivity of these single-spored isolates was evaluated using a detached leaf assay of fungicide-treated susceptible wheat leaf pieces. Five different fungicides, representing three major fungicide classes, were evaluated using 11 concentrations per fungicide. Detached leaves were inoculated with individual isolates and after 10 days leaf pieces were rated for sporulation. Bgt isolates from Mid-Atlantic states (NC, VA), Great Lakes states (MI, NY, OH, PA), and Deep South states (AL, FL, GA, MS) exhibited decreased sensitivity to the Demethylation Inhibitor (DMI) fungicides tebuconazole and prothioconazole compared to isolates from MO, AR, and the Plains states (KS, NE, OK). Plains isolates exhibited decreased sensitivity to the succinate dehydrogenase inhibitor (SDHI) fungicide fluxapyroxad compared to Deep South isolates. Sensitivity to the Quinone outside Inhibitor (QoI) fungicides pyraclostrobin and picoxystrobin did not differ by region. Genotypic evaluation of phenotypically insensitive isolates will further clarify the extent and nature of potentially fungicide-resistant isolates in this collection.

#### **Baseline sensitivities of *Rhizopus stolonifer* and first effective fungicides for management of almond hull rot in California**

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Almond hull rot, caused primarily by *Rhizopus stolonifer* (*Rs*) and sometimes *Monilinia fructicola* (*Mf*), is an increasingly important disease in California almond production. These fungi infect the hull either during late fruit development (*Mf*) or at hull split (*Rs*). Fruit colonization leads to production of toxins that translocate into the fruiting wood, causing twig and branch dieback. Hull rot has been managed culturally by deficit irrigation and by limiting nitrogen application, but this opposes high-density, high-input farming that increases disease pressure. Fungicide treatments add to an integrated management approach. Baseline sensitivities to six fungicides representing DMI (difenoconazole, metconazole), SDHI (fluopyram, fluxapyroxad), and QoI (pyraclostrobin, trifloxystrobin) FRAC groups for inhibition of *Rs* mycelial growth or spore germination were determined using the spiral gradient dilution method. Mean EC<sub>50</sub> values for these fungicides were 0.102, 0.082, 0.292, 0.850, 0.002, and 0.002 mg/L, respectively. Sensitivities to each fungicide were within a ten-fold range, and thus, all isolates are considered sensitive. In field efficacy and timing studies, a single application of these fungicides and selected pre-mixtures at the beginning of hull split timed with a standard insecticide application was sufficient to reduce the disease by 60 to 80% when *Rs* was the primary pathogen. This represents the first fungicide-based field management strategy for hull rot.

#### **Sensitivity of *Neofabraea* spp strain to newly registered fungicides**

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Bull's eye rot, incited by a group of closely related fungi in the genus *Neofabraea*, causes significant economic loss to the Washington State apple industry. The pathogen complex is a significant limitation as it has been listed as a quarantine pathogen with a zero tolerance level for fruit shipped to China and Israel. Access to export markets for Washington State apples relies on the development of effective bull's eye rot control methods. Previous experiments indicate that fungicide treatments containing thiabendazole and thiophenate-methyl provide effective disease control, however use of this treatment is not recommended due to the potential for development of resistance in populations of *Penicillium* spp., which incites the common postharvest disease, blue mold. Fludioxonil (Scholar) did not demonstrate consistently effective control of bull's eye rot in fruit and did not limit *in vitro* growth of the pathogen. Additional analyses were conducted to screen *Neofabraea* spp. isolates for sensitivity to newer commercially available fungicides that include multiple active chemistries. Results from these trials suggest that fungicide containing fluopyram and trifloxystrobin inhibits *in vitro* growth of *Neofabraea* at a level equivalent to that of thiabendazole. In contrast, fungicide treatment containing cyprodinil with difenoconazole, or solatenol alone failed to inhibit mycelial growth or spore germination *in vitro*.

#### **Baseline sensitivities to SDHI fungicides and characterization of the *sdhB* gene in *Venturia inaequalis***

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The succinate dehydrogenase inhibitor (SDHI) fungicides are a class of broad spectrum, single-site fungicides that interfere with fungal respiration in the mitochondrion. The recent registration of next-generation SDHIs for apple scab management prompted an investigation into the baseline sensitivity of *Venturia inaequalis* to penthiopyrad, fluopyram, and benzovindiflupyr. Thirty-five to 70 isolates with no prior exposure to single-site fungicides were used to determine the effective concentration at which conidial germination was inhibited by 50% (EC<sub>50</sub>). Mean EC<sub>50</sub> values for penthiopyrad, fluopyram, and benzovindiflupyr were 0.086, 0.176 and 0.0016 µg ml<sup>-1</sup>, respectively. Linear correlation analysis revealed a significant and positive correlation in sensitivity between fluopyram and penthiopyrad ( $P \leq 0.0001$ ,  $r = 0.66$ ) and fluopyram and benzovindiflupyr ( $P = 0.0014$ ,  $r = 0.52$ ). Genomic sequencing was performed using the Illumina MiSeq platform to identify and characterize the *sdhB* gene for 3 isolates collected from a research, baseline, and commercial orchard population. No common mutation sites commonly associated with SDHI resistance were discovered in these isolates, or in other isolates that were recovered following field applications of SDHIs. Results suggest that SDHIs are highly effective against conidial germination and provide a basis for phenotypic and genotypic monitoring of SDHI resistance in populations of *V. inaequalis*.

### Assessment of QoI Resistance in *Colletotrichum* spp. Isolated from Boxthorn and Apple in Korea

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Strobilurin fungicides including azoxystrobin, kresoxim-methyl, and pyraclostrobin are inhibitors of mitochondrial respiration; they inhibit the cytochrome bc<sub>1</sub> enzyme complex at the Qo site (quinone outside inhibitors, QoIs). QoI resistance has been reported in many pathogens such as *Blumeria graminis* f. sp. *tritici*, *Plasmopara viticola* and so on. The primary molecular mechanism of QoI resistance is a point mutation at codon 143 based on the substitution of glycine by alanine (GGT to GCT, G143A) in the mitochondrial-encoded cytochrome b gene. We examined the sensitivity of *Colletotrichum* spp. isolated from boxthorn fields and apple orchards to pyraclostrobin through the mycelial growth inhibition test on PDA amended with the fungicide. The resistance of *C. acutatum* isolates from boxthorn was determined with values of EC<sub>50</sub> (effective concentration reducing the mycelial growth by 50%). The EC<sub>50</sub> of resistant isolates was more than 1.7 µg/ml. On the other hand, The EC<sub>50</sub> of sensitive isolates of *C. gloeosporioides* from apples was determined as less than 0.24 µg/ml, whereas resistant isolates were more than 66.9 µg/ml. The codon 143 of cytochrome b gene was substituted from GGT to GCT in all of resistant isolates regardless of *Colletotrichum* species. For the successful control of anthracnose in boxthorn fields or apple orchards in Korea, it is required a monitoring of the QoI resistance with an allele-specific PCR with primers generated from the 143 codon substitution.

### Characterization of fungicide resistance phenotypes in *Botrytis cinerea* populations from blueberry in Florida

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*Botrytis* blossom blight caused by *Botrytis cinerea* is an important blueberry disease and is mainly controlled by fungicide applications prior and during bloom. In this study, we evaluated the sensitivity of 624 *B. cinerea* isolates collected from 11 blueberry fields in central Florida between February and May of 2013, 2014, and 2015. Six fields were near strawberry and 5 fields were located 40 km or more from strawberry fields. Isolates were grown on malt yeast extract agar and tested for their sensitivity to pyraclostrobin (Pyra), boscalid (Bosc), penthiopyrad (Penth), fludioxonil (Flud), fenhexamid (Fenh), cyprodinil (Cyp), and iprodione (Ipr) using a spore germination inhibition assay and previously determined discriminatory doses. Overall, disease incidence was higher in samples collected from blueberry fields planted next to strawberry fields compared to blueberry fields alone. In 2013, resistance frequencies were 1.7, 14.9, 46.3, 49.5, 57.8, 57.9 and 92.6% for Flud, Penth, Ipr, Cyp, Bosc, Fenh, and Pyra, respectively. Similar resistance frequencies were observed in 2014 and 2015 with some significant differences across locations and fungicides. Resistance frequencies were higher in blueberry fields next to strawberry fields for all fungicides except Flud, Penth and Ipr. Our findings indicate that resistance to multiple fungicides in *B. cinerea* is widespread in blueberry fields and that resistant populations selected on strawberry may spread to adjacent crops.

### Baseline sensitivity of *Phyllosticta citricarpa* isolates from Florida to succinate dehydrogenase inhibitor fungicides *in vitro* study

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In 2010, Citrus Black Spot (CBS), caused by *Phyllosticta citricarpa*, was first found in Florida. CBS has a negative impact on fresh market fruit and yield production causing fruit lesions and premature fruit drop. This study assessed 116 monoconidial *P. citricarpa* isolates from 'Valencia' and 'Hamlin' with 39 isolates from Collier, 76 from Hendry and 1 from Polk Counties. Isolate sensitivity to the succinate dehydrogenase inhibitor (SDHI) fungicides: boscalid (0, 0.01, 0.1, 1, 5, 10 and 25 µg/ml), fluxapyroxad (0, 0.001, 0.01, 0.05, 0.1, 1 and 5 µg/ml), and fluopyram (0, 0.001, 0.01, 0.1, 0.5, 1 and 5 µg/ml) was evaluated by mycelial growth inhibition assays. The mean EC<sub>50</sub> values for boscalid and fluxapyroxad were 0.179 and 0.026 µg/ml, respectively. These EC<sub>50</sub> values were significantly different for each fungicide ( $P < 0.001$ ). It is expected the EC<sub>50</sub> value of fluopyram will fall between boscalid and fluxapyroxad's values. In the baseline assay all the isolates tested are sensitive to SDHI fungicides, therefore we expect no mutations in molecular analyses. Developing the baseline sensitivity of *P. citricarpa* to SDHI fungicides allow for resistance monitoring. In previous studies, *P. citricarpa* isolates were found to be sensitive to both quinone outside inhibitor (QoI) fungicides and demethylation inhibitor (DMI) fungicides. Thus, SDHI fungicides could potentially be used as rotational sprays with QoI and DMI fungicides to reduce the risk of resistance developing.

### Screening for QoI resistance among *Colletotrichum* species associated with ripe rot of grape in Virginia vineyards

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Ripe rot of grape is a fungal disease caused by two *Colletotrichum* species complexes, *C. acutatum* and *C. gloeosporioides*. This disease can result in significant crop loss and off-flavors in wine. Among recommended fungicides against ripe rot are the QoI group, or strobilurins. We examined the prevalence of QoI insensitivity among *Colletotrichum* isolates collected throughout Virginia during 2013. For the fungicide-amended medium assay, petri dishes containing potato dextrose agar amended with 100 ppm azoxystrobin (Abound, 22.9% a.i. azoxystrobin, Syngenta Crop Protection) and 1,000 ppm SHAM (salicylhydroxamic acid) were inoculated with a mycelial plug of each of the isolates and then incubated at 25°C. The presence or absence of growth on azoxystrobin-amended medium was examined after 6 days. PCR-RFLP was utilized to detect a point mutation (G143A) of the cytochrome b gene. Primers GCCBF1 and RSCBR2 were used to amplify a 120-bp fragment, which was digested with restriction enzyme *FnuAHI* to detect the G143A mutation. The results showed that among 283 isolates tested, 28% of isolates was insensitive to azoxystrobin *in vitro*, and 16% of the isolates had the G143A mutation. The higher percentage with the fungicide-amended medium assay was expected, since there are multiple mutations that can lead to insensitivity. High prevalence of QoI-insensitive isolates in Virginia vineyards warrants the modification of our management recommendations.

### Detection of quinone outside inhibitor resistant isolates of *Erysiphe necator* in Oregon vineyards

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Grape Powdery Mildew (GPM, caused by *Erysiphe necator* (*En*)) is the most persistent disease in the west coast grape industry due to a long and dry growing season which favors its fecundity and inoculum dispersal via airborne conidia. GPM control failures in 2015 led to the exploration of the possibility of quinone outside inhibitor (QoI) fungicide resistance present in Oregon *En* populations. A survey of spatially distinct vineyards in Oregon was performed in which leaf and fruit samples infected with *En* were collected. Isolation and DNA extraction of *En* samples and subsequent quantitative PCR analysis using competitive TaqMan probes detected the presence of the G143A mutation. Isolates with the G143A mutation were considered resistant to QoIs. Conidia germination bioassays using Trifloxystrobin and Kresoxim-Methyl in 1.5% water agar confirmed genetic results of 28 isolates examined. Of the 28 isolates examined, 22 were resistant to both QoIs (EC<sub>50</sub>>100 µg/ml), while 6 were sensitive (EC<sub>50</sub> from 0.004 to 0.06 µg/ml). Using a discriminatory dose of 0.1 µg/ml, 19 of 19 more isolates were resistant to QoIs and agreed with results from the qPCR assay.

### Characterization of resistance to DMI fungicides in *Colletotrichum* spp. isolates from peach

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Isolates of the *Colletotrichum acutatum* species complex were collected from South Carolina and Georgia peach orchards. Phylogenetic analysis of the combined ITS, glyceraldehyde-3-phosphate dehydrogenase, and beta-tubulin gene sequences separated the isolates into two species, *Colletotrichum nymphaeae* and *Colletotrichum fioriniae* subgroups 1 and 2. The sensitivity of these and three other *Colletotrichum* species from peach (*Colletotrichum fructicola*, *Colletotrichum siamense*, and *Colletotrichum truncatum*) to demethylation inhibitor (DMI) fungicides difenconazole, propiconazole, tebuconazole, metconazole, flutriafol, and fenbuconazole were determined. *C. truncatum* was resistant to tebuconazole, metconazole, flutriafol, and fenbuconazole. *C. nymphaeae* was resistant to flutriafol and fenbuconazole. *C. fructicola* and *C. siamense* were sensitive to all DMI fungicides, and *C. fioriniae* subgroup 2 was reduced-sensitive to DMI fungicides compared with *C. fioriniae* subgroup 1. Difenconazole and propiconazole provided the best control efficacy in vitro to all five species. Tebuconazole and metconazole were effective against all *Colletotrichum* species, except for *C. truncatum*. The 14- $\alpha$  sterol demethylase genes *CYP51A* and *CYP51B* from resistant isolates revealed point mutations associated with resistance to DMI fungicides. The strong in vitro activity of some DMI fungicides against *Colletotrichum* species may be exploited for improved anthracnose disease management of peach.

#### **In-vitro azoxystrobin sensitivity of *Colletotrichum gloeosporioides* isolates from blueberry in north and central Florida**

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Anthrachnose pathogens cause destructive diseases of blueberry fruits, leaves, and stems in Florida. Growers in central Florida have reported a lack of efficacy from QoI fungicides, specifically for anthracnose stem cankers on the southern highbush blueberry variety Flicker. Twenty-two single-spore isolates collected from symptomatic stems, leaves, and berries were identified and tested for sensitivity to QoI fungicides. Percent inhibition (PI) of conidial germination on PDA plates amended with 10  $\mu\text{g mL}^{-1}$  of azoxystrobin demonstrated all isolates from central Florida farms were insensitive to azoxystrobin. Isolates from north Florida farms were sensitive. Internal transcribed spacer region (ITS) sequence comparisons grouped all isolates within the *C. gloeosporioides* species complex, and the diverse morphology of the colonies and conidia agreed with this placement. These data suggest the fungicide failures reported by growers in central Florida were due to selection of QoI fungicide insensitivity in stem-infecting populations of *C. gloeosporioides*. Furthermore, insensitive isolates from fruits and leaves indicate that the fungicide azoxystrobin may no longer be a viable management option for ripe rot and anthracnose leaf spot, either. Additional efforts are needed to determine the nature and distribution of the resistance, to develop alternative management options, and to reinforce good resistance management practices for Florida's blueberry industry.

#### **Fungicide sensitivity of *Monilinia vaccinii-corymbosi* in highbush blueberries in Michigan**

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Mummy berry in blueberries, caused by the fungus *Monilinia vaccinii-corymbosi* (*Mvc*), is usually controlled with fungicides, in particular sterol inhibitors which have been used for almost 20 years. Fungicide sensitivity of *Mvc* isolates was studied across Michigan. Isolates were collected from various field sites and grown in pure culture: 72 isolates were tested in poison agar assays using six concentrations of three commonly used fungicides in the sterol inhibitor class (fenbuconazole, metconazole, and prothioconazole). Commercial formulations of these products were used. Colony diameter was measured after an incubation of 14 days at 22 degrees C in the dark and compared to growth on non-amended PDA. EC50 values were calculated for all isolates using a four-parameter logistical curve fit in the computer program R. EC50 values ranged from 0.0016 to 0.0146 ppm for fenbuconazole, 0.0007 to 0.0104 ppm for metconazole, and 0.0021 to 0.0986 ppm for prothioconazole. A slight shift towards reduced fungicide sensitivity was detected in more intensively managed fields compared to baseline sites; however, highly resistant isolates were not detected. The ITS region of the ribosomal DNA of the isolates was sequenced to confirm their identity and assess species-level genetic diversity. All isolates were nearly identical. Although the risk of sterol inhibitor resistance in *Mvc* appears to be relatively low, fungicide resistance management is still imperative.

#### **Induced overexpression of the gene *MfCYP51* may reveal molecular mechanisms associated to tebuconazole resistance of *Monilinia fructicola* in Brazil**

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The aim of this study was to investigate the mechanism associated with *Monilinia fructicola* resistance to demethylation inhibitor fungicide (DMI) tebuconazole in Brazil. In this study 10 isolates (8 DMI-R and 2 DMI-S) with broad tebuconazole sensitivity (0,003 - >10  $\mu\text{g/ml}$ ) were used to quantify the expression levels of the genes *MfCYP51* and *MfABC1*, respectively encoding the target enzyme 14  $\alpha$ -demethylase and mediating the transport of substances across biological membrane. After the mycelial exposure to sub-lethal doses of tebuconazole, the  $2^{-\Delta\Delta\text{CT}}$  method revealed increased *MfCYP51* mRNA levels for 6 out of 7 DMI-R isolates (ranging from 1.06 to 9.08), while DMI-S revealed small expressions levels of 0.14 and 0.46. The induced expression of the *MfABC1* gene with tebuconazole on isolate mycelium not always increased the levels of mRNA. The Mona element which likely trigger the elevated expression of *MfCYP51* was not present on 58 DMI-R isolates from Brazil including those with increased expression, suggesting that other genetic change yet to be discovered may be triggering this mechanism. Full *MfCYP51* sequencing is currently underway. This is the first report on molecular mechanisms for DMI resistance identification for *M. fructicola* isolates from Brazil; providing an important advancement for risk assessment of DMI fungicides used on brown rot management.

#### **Profiling of Resistance to SDHI Fungicides in *Botrytis cinerea* from Strawberry Fields**

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From 2012-13 to 2014-15, a total of 2,570 *B. cinerea* isolates were collected from strawberry fields in the eastern United States and their sensitivity to the SDHI fungicides including boscalid, fluopyram, fluxapyroxad, and penthiopyrad was assessed. Assays were based on visual assessment of presence or absence of mycelial growth on media amended with discriminatory doses of fungicides in microtiter plates. Overall frequencies of isolates resistant to boscalid, fluopyram, fluxapyroxad and penthiopyrad increased over the three years to 30.0, 1.0, 5.5, and 7.4%, respectively. Four resistance patterns designated A, B, C or D were found. Pattern A isolates were resistant to boscalid with the allele H272R at locus *sdhB*; Pattern B isolates were resistant to boscalid and penthiopyrad with the allele H272R or H272Y at locus *sdhB*; Pattern C isolates were resistant to boscalid, fluxapyroxad, and penthiopyrad with the allele H272Y at locus *sdhB*; and pattern D isolates were resistant to boscalid, fluopyram, fluxapyroxad, and penthiopyrad with alleles P225F or N230I at locus *sdhB*. Isolates with alleles H272R, H272Y, N230I or P225F were sensitive to a new SDHIs, benzovindiflupyr, with mean EC<sub>50</sub> values less than 0.5  $\mu\text{g/ml}$  for each genotype, suggesting that this fungicide may be useful for resistance management.

#### **Resistance of *Colletotrichum gloeosporioides* from strawberry to azoxystrobin and reduced-sensitivity to thiophanate-methyl**

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*Colletotrichum gloeosporioides* causes Colletotrichum crown rot (CCR) of strawberry in the southern United States. Symptoms start with wilting of the plants which consequently collapse. A discoloration can be observed when the crown is cut open. CCR is managed with the use of disease-free transplants and fungicide applications. Our objective was to evaluate the sensitivity of *C. gloeosporioides* to two common fungicides used to manage CCR in Florida strawberry fields. Sixty-three *C. gloeosporioides* isolated from strawberry crowns from 1995 to 2015 were evaluated in a conidial

germination assay for sensitivity to thiophanate-methyl and azoxystrobin at discriminatory concentrations of 3 and 100 µg/ml. An isolate collected in 1999 was found resistant to azoxystrobin. After that, resistance was observed sporadically only in isolates collected after 2007. Most of the isolates recently collected during the 2015-16 FL strawberry season were resistant to azoxystrobin. A reduced sensitivity to thiophanate-methyl was observed for all isolates in both fungicide concentrations tested, and none of the isolates was completely sensitive. We conclude that *C. gloeosporioides* isolates resistant to azoxystrobin have been selected after the introduction of this fungicide in the market in 2001. However, it appears that *C. gloeosporioides* isolates from strawberry have a natural reduced sensitivity to thiophanate-methyl.

#### **Increased frequency of multifungicide resistance in *Botrytis cinerea***

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Over four consecutive strawberry production seasons, resistance to 7 chemical classes of fungicides was determined for 2130 isolates from commercial strawberry fields located in South Carolina and 10 other states of the eastern United States. The overall frequencies of isolates resistant to thiophanate-methyl, pyraclostrobin, fenhexamid, boscalid, cyprodinil, iprodione, and fludioxonil increased over the four seasons to 84.7, 73.0, 32.2, 29.9, 29.7, 12.6, and 11.3%, respectively. Resistance to thiophanate-methyl was found virtually in every location. Resistance to fludioxonil was rarely found during the first three seasons, but increased to 11.3% in 2014-15. Our latest data from the 2015-16 season shows the frequency of resistance to fludioxonil has increased to 12.3%, indicating a strong selection for fludioxonil resistance in strawberry fields in the eastern United States. We hypothesize that the more FRAC codes are rotated into the spray program, the more we will select for isolates with increasing numbers of resistance determinants.

#### **Resistance of *Botrytis cinerea* to fungicides in California strawberries**

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*Botrytis* gray mold, caused by *Botrytis cinerea*, is a very destructive pre- and post-harvest fruit rot of strawberry that affects production worldwide. Resistance to foliar applied fungicides has been reported in many regions, including California. The objective of this study is to characterize the fungicide resistance of *B. cinerea* isolates from California. Approximately 800 isolates of *B. cinerea* were collected from four strawberry fruit production regions of California (Oxnard, Santa Maria, Salinas, Watsonville) (10 isolates per ranch, 10 ranches per growing region). Isolates were collected early season (minimal selection pressure) and late season (maximum selection pressure) during the 2015-2016 growing season. Using a previously described technique, sensitivity of each isolate was determined with a discriminatory dosage of each active ingredient: boscalid, cyprodinil, fenhexamid, fludioxonil, fluopyram, iprodione, penthiopyrad, and thiophanate-methyl. Preliminary data show individual isolates with resistance to one or more active ingredients and modes of action. While previous work in California has documented resistance to a few active ingredients in a few fields, this is the first comprehensive study of *B. cinerea* resistance to commonly used fungicides and modes of action. Results of this study will help identify ineffective fungicides and improve gray mold management programs.

#### **Monitoring *Colletotrichum acutatum* Resistance to Quinone-Outside Inhibitor Fungicides in Strawberry**

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*Colletotrichum acutatum*, the causal agent of anthracnose fruit rot (AFR) and anthracnose root necrosis (ARN), is a major fungal pathogen affecting strawberry. Control of AFR and ARN has been dependent on the use of Quinone-Outside Inhibitor (QoI) fungicides. Resistance of *C. acutatum* isolates to QoI fungicides was reported for the first time in 2013 in Florida, and was linked to five specific strawberry nurseries from Canada and North Carolina. In 2016, *C. acutatum* isolates were collected after outbreaks of ARN in Florida and coastal California. Ninety isolates from four nurseries were tested for QoI sensitivity using a discriminatory dose mycelial growth assay. Forty-two isolates were not inhibited at 3 nor at 100 µg azoxystrobin per ml and were considered QoI-resistant. Resistant isolates originated from two of the four nurseries tested. *C. acutatum* resistance to QoI fungicides is becoming a widespread problem in strawberry nurseries located in different regions. This situation warrants immediate changes in anthracnose management strategies that integrate non-chemical strategies to limit selection and prevent spread of QoI-resistant populations.

#### **Sensitivity of *Sclerotinia sclerotiorum* to thiophanate-methyl, fluazinam, and procymidone**

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The sensitivity of 282 Brazilian isolates of *Sclerotinia sclerotiorum* to thiophanate-methyl (TM), fluazinam, and procymidone was assessed using discriminatory doses and concentration that results in 50% mycelial growth inhibition (EC50). No isolate was resistant to fluazinam or procymidone. The EC50 values varied from 0.003 to 0.007 and from 0.11 to 0.72 mg/ml for fluazinam and procymidone, respectively. One isolate collected in 2010 was resistant to TM (EC50 > 100 mg/mL). The 2010 TM-resistant isolate had a L240F mutation in the b-tubulin gene. This is the first report of mutation at codon 240 conferring resistance to benzimidazole in *S. sclerotiorum*. The high-resolution melting analysis allowed the distinction of TM-sensitive and TM-resistant isolates by specific melting peaks and curves. The 2010 TM-resistant isolate had mycelial growth, sclerotia production, and aggressiveness comparable with that of the sensitive isolates. In 2015, 8 isolates were collected from the same region where the 2010 TM-resistant isolate was obtained. All 2015 isolates were sensitive to fluazinam and procymidone, but resistant to TM. The 2015 TM-resistant isolates exhibited considerable instability in mycelial growth rates after consecutive transferrings on PDA. Resistance to fluazinam and procymidone was nonexistent or rare. Resistance to TM may occur, but at this point no robust inference can be made about the persistence of TM-resistant isolates in the field.

#### **Sensitivity of *Sclerotinia sclerotiorum* to iprodione and propiconazole in Bangladesh**

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*Sclerotinia sclerotiorum* is a destructive plant pathogen causing white mold of mustard. The pathogen was first reported in Bangladesh in 2007. Iprodione and propiconazole have been used to control the disease, but the sensitivity of *S. sclerotiorum* isolates to these fungicides in Bangladesh has not been determined. One hundred eighteen *S. sclerotiorum* isolates from Bangladesh and six from Ohio were tested for sensitivity to the two fungicides. Isolates were collected from infected mustard, soybean and cauliflower plants, plated on Potato Dextrose Agar medium (PDA) and purified by mycelial tip culture. Five-millimeter mycelial discs from 2-day-old cultures were plated on non-amended PDA or PDA amended with iprodione (0.0001, 0.001, 0.01, 0.1, 0.3, 1.0, 10.0 or 100.0 ppm) or propiconazole (0.001, 0.01, 0.1, 0.3, 1.0, 10.0 or 100.0 ppm). Cultures were incubated at room temperature and radial mycelial growth was measured after 48 hours of incubation. EC50 (50% mycelial growth inhibition) values were calculated by regression of mycelial growth relative to the control against log10 fungicide concentrations. EC50 values for iprodione and propiconazole ranged from 0.11 to 0.55 and 0.12 to 0.78 ppm respectively. Since *S. sclerotiorum* has appeared recently in Bangladesh and fungicide use has been relatively infrequent, these values will serve as baseline data for monitoring their efficacy going forward.

#### **Presence of fungicide resistant *Botrytis* strains in Norwegian forest nurseries**

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Approximately 30 million seedlings of Norway spruce (*Picea abies*) are planted in Norwegian forests each year, and the main part of these plants are produced by forest nurseries in Norway. Among the greatest challenges in the seedling production is the control of gray mold, caused by *Botrytis*. Fungicides used in Norwegian forest nurseries to control gray mold are thiophanate methyl, fenhexamid, iprodione, and a mixture of cyprodinil and fludioxonil. Increasing disease problems in newly planted seedlings over the last few years, made it necessary to investigate the possible presence of fungicide resistant *Botrytis* strains in forest nurseries. Isolates were collected from spruce seedlings and germ tube growth was measured on nutrient agar amended with different concentrations of the fungicides. We tested fludioxonil and cyprodinil separately, by using fungicides that contained fludioxonil or pyrimethanil (same chemical group as cyprodinil, cross resistance expected) as single active ingredients. Thus far, we have tested 35 isolates, and 54, 26 and 6% of these were resistant to thiophanate methyl, fenhexamid, and iprodione, respectively. Furthermore, 46, 26, 6, and 3% were moderately resistant to thiophanate methyl, pyrimethanil, iprodione, and fludioxonil, respectively. Nine isolates were resistant to two fungicides. We recommend that the nurseries avoid use of thiophanate methyl in the future, and that fenhexamid resistance is carefully monitored.

#### **Fungicide resistance in populations of *Fusarium proliferatum* causing bulb rot of onion in the Pacific Northwest**

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*Fusarium* bulb rot, caused by the fungus *Fusarium proliferatum*, is an emerging disease on onion in Idaho. This bulb rot occurs in storage and shows symptoms similar to neck rot. Symptoms usually start at the neck and progress down along the fleshy scales, but the basal plate is not affected. Infected scales become translucent yellow and water-soaked, later turning tan-brown and soft. Frequently, the rot appears to progress down from the neck along a single fleshy scale in the bulb. In most cases the infected bulbs do not show any pronounced external symptoms or signs of infection. Currently, there are no control measures known for this disease as the mode of infection and conditions required for disease development are poorly understood. Fungicide sensitivity testing was done on a range of fungicides which have shown potential for the control of *F. proliferatum* using spiral plate dilution gradients. Results showed that there was a range of sensitivities in the populations of *F. proliferatum* to the fungicides tested. A knowledge of fungicides showing effective control of this pathogen in vitro is important in the development of effective management strategies to control this disease.

#### **Sensitivity of *Podosphaera xanthii* isolates from Illinois to fungicides**

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Powdery mildew causes more than 50% yield losses in pumpkin and squash fields in Illinois, if it is not managed effectively. Since 2010, growers in Illinois have complained about failure of some fungicides to effectively control powdery mildew in cucurbit fields. This research was conducted to evaluate sensitivity of powdery mildew fungi from Illinois to major powdery mildew fungicide groups, including demethylation inhibitors (DMI), strobilurins or quinone outside inhibitor (QoI), succinate dehydrogenase inhibiting (SDHI), and cyflufenamid. In 2014 and 2015, 34 isolates of powdery mildew fungi were collected from cucurbits fields in 10 counties in Illinois. All of the collected isolates were identified as *Podosphaera xanthii* by using universal fungi primers ITS1F and ITS4 to amplify the internal transcribed spacer (ITS) region of nuclear ribosomal DNA (nrDNA). Twenty of the isolates from different locations and different hosts were tested for their sensitivity to the four fungicides, including triflumizole (DMI), azoxystrobin (QoI), penthiopyrad (SDHI), and cyflufenamid. Our preliminary studies in the laboratory showed that the isolates were highly sensitive to triflumizole, penthiopyrad, and cyflufenamid, but less sensitive to azoxystrobin. The sensitivity of the isolates to azoxystrobin varied. Similarly, severity of powdery mildew in the field plots treated with azoxystrobin was significantly higher than those in the plots received the other three fungicides.

#### ***Podosphaera xanthii* strains with resistance and reduced sensitivity to several fungicides may challenge control of cucurbit powdery mildew**

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Fungicides at risk for resistance development are important for effectively managing powdery mildew (PM) in cucurbit crops because they can move to lower leaf surfaces where the pathogen develops best. Isolates were collected from research and commercial plantings on Long Island, NY, on 16 Sep to 1 Oct 2015, which was after several fungicide applications for PM. A leaf disk bioassay was used to determine fungicide sensitivity. All but 1 of the 57 isolates tested were determined to be resistant to QoI fungicides (FRAC 11) and 79% of the isolates were resistant to boscalid, an SDHI fungicide (FRAC 7). Many grew on leaf disks treated with 500 ppm boscalid as well as on untreated disks. Isolates with very limited or no growth on 500 ppm boscalid disks tended to also be slightly tolerant or sensitive to 40 ppm myclobutanil (FRAC 3) and 40 ppm quinoxifen (FRAC 13); most of these were collected from research plants at LIHREC not treated with any at-risk fungicides (organically-managed and non-treated plots in a fungicide evaluation). Many boscalid-resistant isolates also grew well on disks with 40 ppm myclobutanil and on disks with 40 ppm quinoxifen. Some of these exhibit tolerance to higher doses. These results document strains of *Podosphaera xanthii* with resistance and reduced sensitivity to multiple fungicide chemistries can be selected during one growing season, including where not exposed to all 3 chemistries. Such strains could foil alternation fungicide programs.

#### **Fungicide resistance profiles and virulence of *Stagonosporopsis* species isolates from watermelon and melon in eastern China**

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*Stagonosporopsis* spp. (syn: *Didymella bryoniae*) is a major pathogen on cucurbits. China is the largest producer of watermelon in the world with 22% of the seed imported to the US is coming from China. The purpose of this study was to examine fungicide resistance and virulence profiles of *Stagonosporopsis* spp. isolates in China as a first step in understanding potential global movement of novel strains. Sixty-nine mono-conidial isolates from 4 provinces in east China were collected between 2011 and 2015. Fungicides azoxystrobin, boscalid, difenoconazole, tebuconazole, and thiophanate M were tested using an amended agar assays at the rates of 0.096, 0.034, 0.032, 0.128, and 100mg/L, respectively. Virulence was examined using a detached leaf assay with the watermelon (*Citrullus lanatus*) var: 'Sugar Baby'. Leaves were inoculated with the pathogen and evaluated using a disease scale. Statistical analysis was done using R version 3.2.3 examining frequencies and distributions of fungicide resistance and virulence. All isolates found in East China were pathogenic on a US variety with many isolates having moderate (67%) or even high virulence (29%). In total, 92% of the isolates were resistant to two or more classes of fungicides. Based on these findings, it is apparent that fungicide resistance is present in eastern China and could pose a risk to the global movement of resistant strains.

#### **Investigating dynamics of QoI fungicide resistance in potato early blight complex pathogens in Wisconsin**

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The potato early blight complex (EBC), caused by *Alternaria solani* and *A. alternata*, is a perennial and destructive fungal disease that can lead to loss in yield and quality. Without cultivar resistance, management is with weekly-applied fungicides which can result in pathogen resistance in commercial fields treated with single site mode of action fungicides. One such group is the QoIs, which target the cytochrome *b* of pathogens. In this 2015 study, the F129L mutation which leads to QoI partial resistance was detected in all *A. solani* isolates collected from 4 farms representing unique potato production regions of Wisconsin. Three of the farms conducted conventional production practices; one farm was certified organic. QoI resistance in *A. alternata* was

due to the G143A mutation and was detected in 64% of the isolates in early July, and 91% in late August. At the organic farm, the percentage of *A. alternata* isolates with QoI resistance increased from 0 to 13% over the season. It is possible that *A. alternata*, with a broad host range, may have a wild type preserved in alternative hosts which can move into potato. *Alternaria solani*, which is more specifically a potato pathogen, may have been subjected to QoI selection more thoroughly. Our results suggested that there was rapid selection for QoI resistance within a single production season, indicating the need for modified and integrated management responses for long term management of the EBC.

#### **Sensitivity of *Alternaria* spp. causing diseases in tomato and potato to boscalid and azoxystrobin**

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Tomato and potato growers in Ohio have recently reported reduced efficacy of fungicides used to control diseases caused by *Alternaria*. We aimed to assess the sensitivity of *Alternaria* isolates to two commonly used fungicides. Thirty isolates were collected from symptomatic tomato and potato plants in 2015, 28 of which were identified as *A. alternata* and two as *A. solani*. Isolates were screened for sensitivity to commercial formulations of azoxystrobin and boscalid. Mycelial discs were transferred to potato dextrose agar plates containing 0.0, 0.01, 0.1, 1, and 10 µg boscalid/mL; or 0.0, 0.01, 0.1, 1, 10, and 100 µg azoxystrobin/mL amended with SHAM (100 µg/mL). The diameter of fungal colonies was measured after 8 days of incubation. The effective dose for 50% inhibition (EC<sub>50</sub>) of mycelial growth was calculated for each isolate by regressing the relative growth against the log<sub>10</sub> of fungicide concentration. The *A. alternata* isolates were sensitive or slightly reduced in sensitivity to boscalid. One *A. solani* isolate was highly resistant to boscalid. More than 90% of the *A. alternata* isolates were resistant to some degree to azoxystrobin; both *A. solani* isolates were highly resistant. Overuse of azoxystrobin may have resulted in a shift in *Alternaria* populations to higher resistance to this fungicide. Resistance to boscalid was rare, but fungicide resistance management programs should be followed to reduce the risks of resistance development in *Alternaria* species.

#### **Fungicide sensitivity of *Pythium* species from the North Central Region of the United States**

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Pre- and post-emergence damping-off of soybean in the North Central Region (NCR) can be caused by several *Pythium* spp. Various fungicide seed treatments are used to manage damping-off. Little is known about the effect of temperature on fungicide sensitivity of *Pythium* spp. in the NCR. The goal of this study was to assess fungicide sensitivity of *Pythium* spp. from the NCR to metalaxyl, ethaboxam, and azoxystrobin by assessing mycelial inhibition in vitro at two temperatures. Isolates of four *Pythium* spp. (*P. lutarium*, *P. oopapillum*, *P. sylvaticum*, and *P. torulosum*) from the Midwest, collected through the USDA funded Oomycete Extension Network, were assessed for fungicide sensitivity using five concentrations (0, 0.1, 1.0, 10, and 100 µg a.i./ml) at 13°C and 23°C in an agar medium plate assay. All species from all states, except AR, were more sensitive (>50% mycelia inhibition) to metalaxyl and ethaboxam than to azoxystrobin. At 13°C isolates of *P. oopapillum* from all states, except AR, were less sensitive to all fungicides compared to 23°C. Isolates of *P. sylvaticum* from IA, ND, NE, SD, and WI were less sensitive to all fungicides at 23°C compared to 13°C. At 13°C isolates of *P. torulosum* from IA, IN, ND, NE, and WI were less sensitive to all fungicides tested compared to 23°C. An improved understanding of fungicide sensitivity of *Pythium* spp. could improve our ability to develop and implement effective fungicide seed treatments in the NCR.

#### ***Pythium* species causing damping-off of alfalfa in Minnesota: Identification, pathogenicity and fungicide sensitivity**

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Damping-off and seed rot is an important disease of alfalfa, severely affecting stand establishment when conditions favor the disease. Globally, 15 *Pythium* species cause damping-off and seed rot of alfalfa, although surveys of species causing disease on alfalfa in Minnesota are lacking. Eight species were isolated by a seedling baiting technique from soil of five alfalfa fields in Minnesota with high levels of damping-off. Three species, *P. sylvaticum*, *P. irregulare*, and *P. ultimum* var. *ultimum*, were pathogenic on germinating alfalfa seedlings at 21°C. Strains of seven species isolated from infected soybean, *P. irregulare*, *P. intermedium*, *P. sylvaticum*, *P. recalitrans*, *P. conidiophorum*, *P. ultimum* var. *sporangiiferum*, and *P. ultimum* var. *ultimum*, were pathogenic on alfalfa. Assays with Apron XL (mefanoxam) treated seed showed that fungicide sensitivity varied between and with species with approximately 56% of strains insensitive. In Apron XL amended medium, hyphal density was reduced at all concentrations but all strains had a similar growth rate as on non-amended medium. Insensitivity to Stamina seed treatments (pyraclostrobin) occurred in 94% of strains tested. The presence of broad host range species and fungicide resistance suggests that crop rotation and these widely used seed treatments are not effective tools for managing this disease. These results indicate that resistant cultivars are needed for managing damping-off in alfalfa production systems.

#### **G143A mutation in cytochrome *b* is found in azoxystrobin resistant *Phytophthora cactorum* but not in resistant *Phytophthora nicotianae* from strawberry**

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*Phytophthora cactorum* and *P. nicotianae* cause leather rot of fruit and *Phytophthora* crown rot (PhCR) in strawberry. Leather rot occurs sporadically, but can cause up to 50% fruit loss during conducive weather. In Florida's annual strawberry winter production system, PhCR can be severe resulting in stunting, mortality and severe yield loss. Currently, azoxystrobin is labelled for control of leather rot but not for PhCR. *P. cactorum* and *P. nicotianae* isolates from both leather rot and PhCR were collected from multiple strawberry fields in Florida between 1997 and 2016. Isolates were tested for sensitivity to azoxystrobin at 0, 0.01, 0.1, 1.0, 10, and 50 mg/L amended with SHAM (100 mg/L). The isolates were separated into two groups, sensitive isolates, with EC<sub>50</sub> values of less than 1.0 mg/L, and resistant isolates having EC<sub>50</sub> values greater than 50 mg/L. Resistant *P. cactorum* and *P. nicotianae* isolates were found among both leather rot and PhCR isolates collected after 2010. The first 450 bases of the mitochondrial cytochrome *b* gene were sequenced from a selection of resistant and sensitive isolates of both species. The G143A mutation reported to cause azoxystrobin resistance was found in all three resistant *P. cactorum* isolates but not in resistant *P. nicotianae* isolates or in any sensitive isolate. This is also the first report of resistance to azoxystrobin in *P. nicotianae* from strawberry.

#### **Prevalence of metalaxyl resistance in *Pythium* populations in dryland agriculture in the US Pacific Northwest**

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Metalaxyl (mefenoxam) has been the primary fungicide used in managing *Pythium* damping off of chickpea and other pulse crops. Metalaxyl resistance among *Pythium* spp. has been reported from the irrigated Columbia basin in the state of Washington where metalaxyl has been applied multiple times annually to manage potato diseases, but not from dryland agriculture where metalaxyl has been used only once per year in seed treatments. However, in the spring of 2014 and 2015, severe seed rot and damping-off of metalaxyl-treated chickpea seeds were found in the dryland agriculture region in southeastern Washington and northern Idaho, and were associated with *Pythium* isolates with high levels of resistance to metalaxyl. High densities of metalaxyl-resistant *Pythium* populations were found in areas of severe chickpea seed rot and damping-off, and were observed in seven commercial chickpea fields in southeastern Washington and northern Idaho. *P. ultimum* var. *ultimum* dominated the populations that were highly resistant to metalaxyl. ED<sub>50</sub> values for metalaxyl-resistant isolates were significantly higher than those of metalaxyl-sensitive isolates. Under controlled conditions,

metalaxyl treatments failed to protect chickpea seeds from seed rot and damping-off following inoculation with metalaxyl-resistant *Pythium* isolates. An effective alternative measure is urgently needed to successfully manage metalaxyl-resistant *Pythium* in chickpea production.

#### **Screening for phenylamide fungicide insensitivity in Wisconsin hop downy mildew (*Pseudoperonospora humuli*) populations**

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*Pseudoperonospora humuli* is the oomycete pathogen causing hop downy mildew, an important disease concern in WI. The disease is primarily managed by fungicides since varietal resistance is unavailable. Phenylamides have been shown to be systemic and highly effective in control, but due to resistance, particularly in the Pacific Northwestern (PNW) hop producing region, their use has dwindled. To date, the use of phenylamides, namely mefenoxam, has been extremely limited in WI due to the high cost and uncertainty of efficacy. In 2015, nineteen *P. humuli* isolates were collected from a commercial hop yard in Dane County, WI and screened for mefenoxam insensitivity using a leaf disk assay. Fourteen leaf disks were placed onto either 1% water agar (WA) or 1% WA amended with 25 µg/ml of mefenoxam (Ridomil Gold SL, Syngenta). The disks were inoculated at 3 sites with sporangial suspensions from each isolate and the incidence of sporulation at each site was tallied after 5-7 days. The percentage of total sporulating sites was calculated and isolates were considered insensitive if the incidence of sporulation on amended media was at least 50% of that on nonamended media. All isolates showed no sporulation on WA plates amended with mefenoxam, and were sensitive. Mefenoxam may be highly effective in managing hop downy mildew in this location. Additional locations will be tested to better assess the phenylamide sensitivity status and aid in grower fungicide decision-making.

#### **Production of *Phytophthora nicotianae* isolates resistant to oxathiapiprolin**

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Black shank, caused by *Phytophthora nicotianae* (*Pn*), is a devastating disease of tobacco throughout the world. Oxathiapiprolin (OXA), a newly discovered fungicide, has shown efficacy against numerous oomycetes including *Pn*, but the potential for OXA resistance is unknown. The generation of resistance to OXA was attempted using UV light mutagenesis and mycelial adaptation with four *Pn* isolates. UV mutagenesis generated two isolates (EdgeB7-M1 and YadA28-M1) with mycelial resistance to OXA. Sporangia production of both UV mutants also showed increased insensitivity to OXA. Resistance produced by UV mutagenesis was stable after five consecutive transfers on non-amended medium. In greenhouse studies, EdgeB7-M1 remained pathogenic to tobacco, while YadA28-M1 was nonpathogenic. For mycelial adaptation, 48 colonies, twelve replicates from each of the four isolates, were transferred fifteen times on OXA medium. After the 15<sup>th</sup> transfer, twelve colonies from the initial 48 had a significant increase in mycelial insensitivity to OXA, compared to the wild-type isolates. Increased insensitivity from mycelial adaptation was not stable. Sixty-six percent of the isolates lost their insensitivity after fifteen successive transfers on a non-amended medium. Of the twelve adapted isolates, one remained pathogenic on tobacco. Our results show that the generation of OXA resistant *Pn* isolates is possible; however resistance may be associated with fitness penalties.

#### **Management of mefenoxam-resistant isolates of *Pythium ultimum* causing pythium leak on potato in the Pacific Northwest**

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Mefenoxam is a fungicide used to manage oomycete pathogens on potato. Resistance to mefenoxam has developed in *Phytophthora infestans* in all potato growing regions of the U.S. and is becoming more common in *P. erythroseptica*. In a 2006 survey, many fields in the Columbia Basin were found to have mefenoxam-resistant isolates of *P. ultimum*. The percentage of resistant isolates to sensitive isolates for these fields ranged from 0 to 92.5%. Despite the risk of resistance and the cost of mefenoxam, it is still widely used in potato production in the Pacific Northwest (PNW) due to its efficacy in managing pythium leak and the lack of effective alternative compounds. In the PNW, pythium leak is most often seen at harvest or during storage. However in recent years, particularly in fields with high levels of resistance, extensive damage has been found weeks ahead of harvest, suggesting a higher disease risk for resistant isolates than sensitive ones. In this study, a field survey was undertaken to evaluate the extent and persistence of mefenoxam resistance in populations of *Pythium* in the PNW and determine potential alternative fungicides that can be used for the control of mefenoxam resistant *P. ultimum* isolates. Results showed that the levels of mefenoxam resistance in PNW *Pythium* isolates had decreased since 2006. Several new fungicides were also identified which had good efficacy against *P. ultimum*.

#### **Oxathiapiprolin baseline sensitivity distribution of *Phytophthora capsici* isolates**

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Oxathiapiprolin is the only active ingredient in a new class of fungicides (piperidinyl-thiazole-isoxazoline) that is active against downy mildews and *Phytophthora* species, and generally weak against *Pythium* species. It is active against multiple developmental stages of Oomycete fungi including inhibition of mycelial growth, sporangial germination, zoospore release, and zoospore germination. The proposed site of action is the inhibition of an oxysterol binding protein (OSBP). FRAC has assigned oxyathapiprolin to group U15 and is considered to have a medium to high risk for resistance development. The oxathiapiprolin baseline sensitivity of *Phytophthora capsici* has been constructed using 40 isolates never exposed to this fungicide. *P. capsici* isolates were obtained from pepper, zucchini, squash, tomato, watermelon, pumpkin, smooth gourd, bitter melon and an unknown host. Sensitivity tests were conducted *in vitro* using V8 agar media amended with oxathiapiprolin. Mycelial growth inhibition was quantified using the following oxathiapiprolin concentrations: 0, 0.000001, 0.00001, 0.0001, 0.001, 0.01 and 0.1 mg.ai/L. The baseline sensitivity distribution (ED50 values) of the *P. capsici* isolates ranged from 0.00006090 to 0.0005169 with a geometric mean of 0.00019716 mg oxathiapiprolin/L and a range of 8.5X. Oxathiapiprolin baseline information is critical for the early detection of resistant isolates and the establishment of effective resistance management recommendations.

#### **Fungicide sensitivity of *Phytophthora infestans* isolates collected in Colombia**

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*Phytophthora infestans*, is a major constraint to potato production in Colombia. The disease is present in all regions where potatoes are grown and may cause losses of around 265,000 tons of potatoes per year. Control of late blight in Colombia is mainly achieved by the use of fungicides. Due to the specific mode of action of some of these fungicides and the high selection pressure exerted by the continued use of these products it is likely that some *P. infestans* strains in Colombia have already developed resistance against some of these systemic fungicides. Therefore, the aim of this study was to determine the sensitivity of isolates collected in potato-producing regions of Colombia to the systemic fungicides dimetomorph, fluopicolide, and propamocarb. Baseline sensitivity was tested *in vitro* on pea agar media amended with a range of concentrations of each fungicide tested. Dose-response curves and values for the median effective concentration, at which 50% of mycelial growth was inhibited, were determined for each isolate.

#### **Assessment of acquired resistance of originally sensitive isolates of *Phytophthora infestans* to the systemic fungicides fluopicolide and propamocarb**

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*Phytophthora infestans*, a plant pathogenic oomycete, is the causal agent of late blight disease of potatoes and tomatoes. This disease causes devastating economical losses each year and control is mainly achieved by the use of fungicides. Previous studies have reported that sensitive isolates to the phenylamide fungicide, mefenoxam, become tolerant after a single passage on mefenoxam-containing medium. The acquired resistance of the sensitive isolates appeared to have a cost on the rate of mycelial growth and declined slightly with subsequent culturing on medium free of mefenoxam. The aim of this study was to investigate whether the same phenomenon is observed using two different systemic fungicides: fluopicolide and propamocarb. Susceptible isolates of *P. infestans* were exposed to three different concentrations of fungicide-amended medium: 0, 5, and 10 µg ml<sup>-1</sup> for fluopicolide and 0, 1 and 100 µg ml<sup>-1</sup> for propamocarb. To assess if the isolates had acquired resistance to each of the fungicides tested, mycelia from isolates growing at 5 µg ml<sup>-1</sup> for fluopicolide and 1 µg ml<sup>-1</sup> for propamocarb were transferred once again to media with the same concentrations mentioned above. This study provides further insights into the *in vitro* adaptability of *P. infestans* against antifungal stress.

#### **Fertigation and soil acidification sustain root density of Huanglongbing-infected trees in Florida citrus groves**

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Huanglongbing (HLB) is a devastating citrus disease caused by the phloem-limited bacterium *Candidatus Liberibacter asiaticus* (Las). Early symptoms of HLB include fibrous root loss and leaf blotchy mottle, followed by yield decline, leaf drop, and canopy dieback. As a consequence of initial bacterial infection of fibrous roots, a 30-50% reduction in fibrous root density and elevated soil *Phytophthora* populations were detected in field surveys. Continued sampling of Hamlin and Valencia orange trees on Swingle citrumelo rootstock in different stages of HLB decline revealed that root loss occurs in two stages. The second phase of root loss (70-80%) begins at the early stage of tree canopy thinning resulting from leaf drop and branch dieback. Managements have been implemented to reduce soil, nutrient and water stress, and *Phytophthora* root rot. They include frequent irrigation cycles, fertigation and acidification of irrigation water and soil to reduce rhizosphere pH, and fungicides. Root density of trees under these practices fluctuates seasonally and annually but has not declined over the last 3 years. Trees managed with soil acidification and fertigation have steadily recovered in health and yield.

#### **Status of Huanglongbing (HLB)**

##### **(citrus greening) Multi Agency Coordination (MAC) projects on thermal treatment of citrus trees with citrus greening**

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Citrus trees infected with '*Candidatus Liberibacter asiaticus*', subjected to heat treatments, have shown an improvement in citrus yields and vigor. Thermal treatment equipment for all sizes of citrus trees is being developed and improved for citrus trees infected with '*Ca. Liberibacter asiaticus*'. Two MAC projects are contributing to the development and improvement of thermal treatment equipment for all sizes of citrus trees infected with this pathogen. Researchers involved in the first project have developed a mobile physical system using steam to prolong the productive life of citrus trees eight feet tall and smaller. This system uses a tent canopy that goes over the tree followed by steam treatment. Second project has developed a mobile thermotherapy system for citrus trees as tall as thirty feet. The entire system supports a tent canopy that continuously treats as it progresses in the row without stopping the steam treatment. Both projects have successfully heat-treated grove trees infected with HLB. There have been improvements in the thermal equipment to provide a fully functional mobile steaming system for immediate use on all sizes of trees for commercial treatments.

#### **Canopy and root response of HLB-affected citrus trees to steam-generated thermotherapy**

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Thermotherapy is an old technique used to control plant pathogens. Citrus Huanglongbing (HLB) threatens Florida's multibillion dollar citrus industry and has no effective control measures. The phloem-limited bacterium, *Candidatus Liberibacter asiaticus* (Las), is the causal agent of HLB and infects both canopy and root system. Thermotherapy can reduce Las titer in infected trees and this study explores steam as a heat source for rapid treatment to extend the productivity of HLB-affected citrus trees. Optimum temperature-time duration is important to reduce Las titer without affecting tree productivity. A randomized split-plot design field trial was laid out with the main plots at 3-month intervals to determine optimum time-of-year to reduce Las titer and the subplots at 6 temperature-time combinations (58°C/15s; 58°C/60s; 58°C/90s; 58°C/120s; 60°C/30s and untreated control, UTC) with 3 replications per treatment. Four leaves/tree from 10 trees/rep were collected quarterly and tested for Las titer via standard qPCR. Roots were assessed for Las titer as roots are an untreated reservoir. Preliminary data show an overall reduction in titer a month after the July treatment. Las titer increased in all leaves, but more in the UTC than the treatments, whereas root Las titer was reduced more in treatments than the UTC. This may be due to change of phloem flow from root to the treated canopy along with Las. Seasonality may be critical for successful suppression of Las.

#### **Inoculum sources of *Xanthomonas fragariae* in strawberry nursery packing houses: Presence, viability and transmission**

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*Xanthomonas fragariae* causes strawberry angular leaf spot, an important disease in strawberry nursery production. When plants are dug and harvested in the field, they are transported to a "trim shed" where the leaves are removed and the roots are trimmed with table-mounted steel blades. Maintaining constant and stringent phytosanitary conditions is nearly impossible because of the sheer numbers of plants handled each day as part of this sorting and packaging process. To determine the likelihood of *X. fragariae* transmission during this process, PBS-soaked cotton balls were used to swab the blades and tables at nursery trim shed stations after processing strawberry plants harvested from infected fields. *X. fragariae* presence and percent viability were determined by squeezing PBS from the cotton balls and testing it by PMA-qPCR. The likelihood of disease transmission was estimated by rub-inoculating strawberry plants with the cotton balls. *X. fragariae* was detected in ~70% of the samples and ~30% of these contained living bacteria. Disease symptoms were observed in 9 of 62 strawberry plants inoculated. Results indicate that contamination of healthy plants is likely to occur during the post-harvest trimming process in nurseries and identified points where sanitation can be improved for better disease control.

#### **Assessment of *in-vitro* efficacy of different disinfectants against common bacterial diseases on woody ornamentals**

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Plants are continuously exposed to disease causing agents, which enter to the nurseries in many ways. Cutting tools and other equipment that are used to work with infected plants is considered as a major source for bacterial disease spread. Various products are available in the market to use as disinfectants to clean cutting tools. This study was conducted to evaluate the effect of commercial disinfectants on *Xanthomonas axonopodis*, *X. campestris* and *Pseudomonas syringae* pv. *syringae*. We determined the contact time required to kill those bacterial pathogens by disinfectants in different chemical classes. The relative shelf-life stability of disinfectants was also determined. Replicated *in-vitro* experiments were conducted in which pure cultures of bacterial pathogens were exposed to disinfectants for 1, 30 and 60 sec, then 100 µL aliquots were withdrawn, plated on NBYA medium, and incubated at 25°C for 3 days. Clorox regular bleach (10%), Virkon-S (0.5, 1 and 2%), Last rinse sanitizer-beer clean, Seventh generation multi surface, Seventh

generation daily shower, Seventh generation all purpose, alcohol (70%), vinegar (100%), Lysol, ZeroTol 2.0 and Green-Shield killed all three pathogens at all exposure times during 3 weeks shelf-life of products. Further studies will be done to determine efficacy of these disinfectants in preventing plant-to-plant spread of bacterial diseases through cutting tools.

#### **Effective treatments for eradication of *Xanthomonas cucurbitae* in pumpkin seed**

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Bacterial spot, caused by *Xanthomonas cucurbitae*, has become a serious disease of pumpkin in Illinois and other states in the Midwest. *X. cucurbitae* infects leaves and fruit. Fruit infection often results in fruit rot. More than 90% of fruit infection with *X. cucurbitae* in pumpkin fields has been reported. *X. cucurbitae* is considered as a seed-borne pathogen, hence developing an effective seed treatment to eradicate the pathogen in seed is essential. This study was conducted to develop reliable treatments for eradication of *X. cucurbitae* in seed. Hot-water, HCl, and NaClO treatments for eradication of *X. cucurbitae* in 'Howden' and 'Dickinson' pumpkins were assessed. Seed were treated in water with 49, 50, 51, 52, 53, 54, 55, and 56°C, for 5, 10, 15, 20, 25, and 30 min at each of the temperatures. Treatment at 55°C for 10 min eradicated *X. cucurbitae* in both naturally-infected and artificially-inoculated seed without significant adverse effects on seed germination or seedling vigor. HCl treatments included 0.5, 1.0, and 2.0% HCl solutions for 10, 20, 30, 40, 50 and 60 min. The most effected HCl-treatment was 0.5% concentration for 40 min, which eradicated *X. cucurbitae* in the seed without any negative effect on seed germination or seedling vigor. For NaClO treatment, pumpkin seeds were soaked in NaClO solution with concentration of 0.5, 1, 1.5 and 2% for 1, 2, 3 and 4 min. None of the NaClO treatments completely eradicated *X. cucurbitae* in pumpkin seed.

#### **Silicon Augments *Xanthomonas gardneri* symptoms in Tomato cv. Early Girl**

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Silicon (Si) is a plant nutrient that enhances stress tolerance. Tomato accumulates a moderate amount of Si in foliar tissue, but its effect on *Xanthomonas gardneri* infection has not yet been determined. To test this, 'Early Girl' tomatoes were grown hydroponically in nutrient solution with (Si+) or without (Control) 2 mM potassium silicate. Plants were dip-inoculated with *X. gardneri*. Symptoms developed within 24 hrs post-inoculation. Total symptomatic leaf area was quantified 10 dpi, using the Assess 2.0 Program. Si-treated plants had significantly more lesions and higher symptomatic leaf area (11.7%) compared to controls (6.5%). Symptoms present on Si+ plants were spread throughout the leaf, while lesions on control plants were concentrated around the leaf margins. This suggested entry by *X. gardneri* in Si+ plants was through both stomates and hydathodes, while control plants were infiltrated mainly via hydathodes. To determine if differences in symptom formation between Si+ and control plants was simply due to changes in stomatal opening, stomatal conductance was directly measured between Si+ and control plants that were removed from hydroponics solution for 2 min. At 570s, stomatal conductance in Si+ plants compared to control was slightly, but significantly lower. This suggests that differences in *X. gardneri* symptoms in 'Early Girl' are not due to changes in the stomatal conductance influenced by the dip-inoculation method.

#### **Study of the influence of winter rye on soybean seedling and root rot diseases**

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Cover crops can enhance or suppress plant diseases, but little is known about the effect of cover crops on soybean diseases. In 2015, the effect of winter rye (*Secale cereale* L.) cover crop on soybean seedling and root rot was studied at two experimental sites, Boyd and ISUAG-USB, in Ames, IA. Both sites were under a no-till corn-soybean rotation. Cover crop treatments were rye and no rye, at Boyd; and early termination rye, late termination rye, and no rye, at ISUAG-USB. Variables assessed were soybean stand, root rot severity, dry plant weight, healthy green leaf area (HGLA), and yield. Root rot severity was visually assessed on a percent scale, and HGLA was measured as canopy percent reflectance using a hand-held radiometer. At Boyd, soybean stand ( $P=0.68$ ) and yield ( $P=0.61$ ) were not affected by treatment, but healthy green leaf area (HGLA) was higher in rye plots ( $P<0.001$ ) compared to plots with no rye. At ISUAG-USB, no rye plots had more soybean stand ( $P=0.08$ ), greater yield ( $3105 \pm 304$  kg/ha) ( $P=0.07$ ), and higher HGLA ( $P<0.001$ ) compared to plots with late termination rye. Early termination rye did not differ from late termination and no rye treatments for any variables. No difference in dry plant weight and root rot severity were observed between treatments at both sites ( $P>0.2$ ). Site variables, as well as cover crop treatments, can influence soybean crop response. This study is going to be repeated in 2016, and additional research is underway to quantify *Fusarium* and *Pythium* populations at both sites.

#### **Reduced *Macrophomina phaseolina* colonization of soybean by supplementing with the secondary nutrients calcium and magnesium**

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*Macrophomina phaseolina* (Tassi) Goid (*Mp*) is a ubiquitous soilborne fungal pathogen that causes charcoal rot (CR) of soybean. Charcoal rot annually limits profitability, especially in years when hot and dry environmental conditions persist. Throughout the southern United States, CR consistently remains one of the top five soybean diseases and has resulted in an estimated 11 million metric ton reduction in yield over the past 20 years. The objective of this research was to reduce *Mp* colonization of field grown soybean by supplementing secondary nutrients, specifically calcium (Ca) and magnesium (Mg). In 2014 and 2015 non-irrigated, *Mp*-inoculated field trials were conducted with a CR-susceptible and a CR-moderately-resistant cultivar. Treatment applications consisted of 1,120 kg/ha rate of Ca and Mg alone and in combination at each of three timings: pre-plant, at plant, and pre-plant fb at plant. Control plots consisted of a completely non-treated non-inoculated in addition to an *Mp*-inoculated non-treated. Root samples were taken at the R3, R5, R7, and R8 growth stages. Colony forming units (cfu) were enumerated from ground, disinfested root tissue using a semi-selective medium. Reductions in cfus were observed to be approximately 35% in the moderately-resistant cultivar and a 45 to 50% reduction in the susceptible cultivar, compared to the non-treated. Reduction in colonization was observed in some cases although not across all treatments.

#### **Evaluating biodegradation treatments for the reduction of inoculum and incidence of citrus black spot *Phyllosticta citricarpa* in Florida citrus groves**

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The fungal disease citrus black spot (CBS) has become an increasing problem in Florida citrus groves. We conducted a 3-year study to evaluate the effect of 5% urea (9.4 kg/190 l water/0.4 ha), Soil-Set (142 ml/190 l water/0.4 ha), and a water control on the level of the *P. citricarpa* or the morphologically similar citrus endophyte *P. capitalensis* in the leaf litter and subsequent CBS incidence on fruit in a Valencia grove in Immokalee, FL. The experiment was a randomized complete block design with three rows per treatment (trt) and four blocks. Leaf litter samples were collected from arbitrarily selected trees at time of treatment and then three times biweekly post-treatment application. For every collection, 100 leaves/trt/block were inspected for fungal sporocarp presence and each fungus was quantified via qPCR. The disease incidence on fruit was collected for 50 fruit/tree on 125 trees/trt/block. Fungal DNA levels were analyzed with a 3-way ANOVA and fruit disease incidence by contingency table analysis and chi-square tests. Leaf litter DNA had a significant interaction ( $P < 0.0001$ ) between block x trt x collection date. For the incidence of disease on fruit, significant

treatment effects were seen in 2015 ( $P = 0.002$ ) and 2016 ( $P < 0.0001$ ) with Soil-Set reducing disease incidence by 29.6% and 26.5%, respectively but not with urea. Soil-Set may enhance the efficacy of fungicide applications for the management of CBS.

#### **Utility of compost tea for disease management in grapes**

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Compost tea is a fermented watery extract of compost used for disease suppression and plant nutrition. A replicated trial was conducted in a 'Niagara' vineyard in Clarksville, Michigan to evaluate the efficacy of compost tea for disease control. Non-aerated manure-based compost tea was prepared over a 2-wk period, and foliar applications were made with a handheld sprayer every 7 or 14 days from 10 June through 27 Aug, 2016. Undiluted compost tea with NuFilm-P adjuvant applied every 7 days provided good control of black rot, powdery mildew, downy mildew, and Phomopsis cane and leaf spot. In general, less frequent and more dilute applications were less effective. 'Niagara' leaves were sampled daily for 7 days after application of aerated manure-based compost tea. Bacterial populations were higher on leaves sprayed with compost tea compared to non-treated leaves but declined to almost background levels after a week. Both yeast and fungal populations were reduced immediately after the application, but later increased over the control. Bacteria from various manure- and plant-based compost teas were isolated and evaluated for antagonism against *Botrytis cinerea* by dual plating. The majority of isolated bacteria belonged to the Bacillaceae family as determined by 16s rRNA sequencing. This study shows that compost tea may be used for disease suppression in grapes but further evaluation is warranted.

#### **Evaluation of disinfectant treatments to reduce transmission of *Didymella bryoniae* during watermelon grafting**

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Gummy stem blight can develop in greenhouses on watermelon seedlings grown as transplants or as scions and on cucurbit rootstock seedlings used in grafting. When diseased seedlings are cut during grafting, the pathogen, *Didymella bryoniae* (*Stagonosporopsis* sp.), may be transferred to healthy seedlings. The objective of this study was to evaluate disinfectant treatments to prevent or reduce transmission. In a first experiment, Kleengro (didecyl dimethyl ammonium chloride), Physan 20 (benzyl ammonium chloride) and Virkon S (potassium peroxymonosulfate) were ineffective when sprayed onto entire watermelon seedlings before or after cutting hypocotyls with a blade contaminated with mycelium or conidia. In a second experiment, applying 0.6% sodium hypochlorite, 0.4% Physan 20 or 70% and 95% ethanol to a contaminated blade or heating it before cutting watermelon hypocotyls reduced incidence of gummy stem blight ( $P=0.01$ ); water, 3% hydrogen peroxide, 1% Virkon S and 7.5% Kleengro were ineffective. In a third experiment, both the watermelon scion and the interspecific hybrid squash rootstock were cut with contaminated and treated blades before grafting. After 7 days in a humid healing chamber, heating the contaminated blade reduced incidence (11%) more than ethanol (45%), and both treatments reduced incidence compared to Physan 20 (100%) and water (100%) ( $P=0.01$ ). Using heat or ethanol to disinfect cutting tools may reduce transmission of *D. bryoniae* during grafting.

#### **Scab severity in relation to hedge pruning pecan trees in the Southeastern USA**

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Scab is the most damaging disease of pecan in the Southeastern USA. Pecan trees are tall (up to 30+ m), and managing disease in the upper canopy is problematic. Hedge pruning trees to ~12 m is being explored to facilitate efficacy of ground-based fungicide sprays, but resulting vigorous shoot growth could result in more severe scab. Nine experiments compared scab severity on trees receiving hedge pruning regimes vs no hedging. All trees received fungicide treatments (ground-based air-blast sprays and up to 3 aerial applications). Results showed that different hedging methods either had no effect, increased or decreased scab severity on immature fruit in one experiment ( $P<0.0001$ , assessed before mid-August [immature fruit were assessed in 8 of the 9 experiments]), and on mature fruit in 6 of 9 experiments ( $P<0.0001$ , assessed after mid-August), but in all cases differences were small. However, height in the canopy invariably had a significant effect on scab severity, with up to 18-fold more severe scab on fruit at  $\geq 12$  m compared to 5 m. Immature fruit high in the canopy had more severe scab in all experiments ( $P<0.0001-0.03$ ), and mature fruit had more severe scab in 6 of 9 experiments ( $P<0.0001$ ). In trees that received the same fungicide program the effect of hedging in increasing scab severity was negligible and not consistent, but scab was consistently more severe at greater heights in the canopy of both hedge pruned and non-hedged trees.

#### **Potential of heat treatment for management of *Botrytis cinerea* resistance on strawberry**

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*Botrytis cinerea* is the causal agent of gray mold, an important disease of strawberry in Florida. The use of fungicides to control gray mold has been exploited over the past years and as a result, resistance to many fungicide groups has been reported. In this study, we are aiming to determine the effect of a heat treatment that has proven to reduce systemic infection with *Xanthomonas fragariae* in strawberry propagating material on the survival of fungicide-resistant *B. cinerea* isolates. For this, four different *B. cinerea* isolates were used to evaluate survival at four temperatures (44, 48, 52 and 56°C) and seven different duration times (1, 5, 10, 30, 60, 120 and 240 min). After treatment, the spores were transferred to a growth medium to determine the percentage of spore survival. Although, *B. cinerea* spores survived for 1 min for all temperatures, germination was reduced by 28 to 50% at the highest temperature. Spores of all isolates exposed to 44°C for 30 min did not survive and spore germination was reduced by 7 to 16% between 48 and 52°C for 1 min, with no spores survived for all other duration times. Previous experiments have determined that most strawberry cultivars can tolerate 44°C for up to 4 h. Thus, heat treatment of strawberry plants is a feasible strategy to produce disease-free strawberry plants and to reduce fungicide-resistant inoculum.

#### **Eradication of the boxwood blight pathogen, *Calonectria pseudonaviculata*, in compost**

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*Calonectria pseudonaviculata*, the causal agent of boxwood blight, is a serious threat to boxwood growers across the country. Boxwood blight is currently present in at least 15 US states, however this number is likely to increase as more infections are found. Due to the production of microsclerotia by the pathogen, there is concern that if infected plants are composted, the pathogen could survive the composting process and possibly cause further infections if the compost is used around healthy boxwoods, or other susceptible plants. To determine survivability during the composting process, discrete microsclerotia were inoculated into compost within a bioreactor system and subsequently sampled at set time/temperature intervals. Survival was rated as a plus/minus system based on the presence of microsclerotia germination. The data presented here are a refinement of data previously presented previously, and suggest that *C. pseudonaviculata* microsclerotia will not survive composting at greater than 50°C for over 24 hours. Further work needs to be done to define the minimum time/temperature that is sufficient to ensure complete eradication. In addition, similar research is needed to assess pathogen survival within infected plant material and to confirm the time/temperature combinations *in vivo*.



### **Thermal inactivation of *Calonectria pseudonaviculata*, the causal agent of boxwood blight**

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Thermotherapy is an effective method for eliminating viruses and microorganisms from seeds and bulbs, but little information is available on the use of this approach to inactivate pathogenic fungi in leaves of woody plants. In this study, we investigated the utility of hot water treatments for killing *Calonectria pseudonaviculata* (Cps), a fungal, foliar pathogen of boxwood (*Buxus*) during propagation. Experiments predicting the thermal death kinetics of Cps conidia, and experiments determining the ability of commonly grown, commercial boxwood cultivars to survive exposure to hot water were performed. Conidia of three field isolates of Cps were suspended in water and exposed to temperatures ranging from 45°C to 55°C for 0 to 20 min. Suspensions were then spread on potato dextrose agar, and percent spore germination was assessed after 24 h of incubation. Approximately 16 min were required to kill 90% of conidia exposed to 45°C, but only 4.5 min were required to kill 90% of spores when heated to 55°C. Cuttings from six partially resistant boxwood cultivars were submerged in water heated to 45°C, 50°C, and 55°C for 0 to 60 min. Treated cuttings were placed under mist and their rooting responses were rated 86 to 91 d later. The ability of boxwood cuttings to produce roots after hot water treatment at 45°C and 50°C varied by cultivar, whereas no cultivars survived treatment at 55°C. Further experiments will determine whether hot water can eliminate the pathogen *in planta*.

### **Using soil amendments to reduce survival of *Botrytis cinerea* sclerotia in Mid-Atlantic diversified farming systems**

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*Botrytis cinerea* causes major pre-harvest losses in a wide range of crops in the Mid-Atlantic, including strawberries, blueberries, raspberries, and grapes. Management challenges have escalated with the loss of soil fumigants as well as increased land-use intensity associated with crop diversification. In a summer 2015 field survey, Maryland fields had up to 350 *B. cinerea* colony forming units per gram of soil and 20% yield losses from gray mold. The objective of this project was to evaluate the efficacy of soil amendments in degrading *B. cinerea* sclerotia – the most resilient source of inoculum. In potted studies, sclerotia viability decreased in crab meal and leaf compost treatments by 91.7% ( $\pm$  5%) and 96.7% respectively, compared to 71.7% ( $\pm$  5%) in untreated soil sixty days after burial. Percent of sclerotia colonized by hyphal fungi was highest in the crab meal (86.7%  $\pm$  3.3%) and leaf compost treatments (93.8%  $\pm$  6.3%). Similar trends between sclerotia viability and colonization were seen in Maryland field trials: sixty days after burial, the leaf compost + crab meal treatment had the lowest sclerotia viability and the highest colonization by bacteria or yeasts. These studies suggest that soil amendments may hold promise in reducing *B. cinerea* survival and dispersal in diversified farming systems in the Mid-Atlantic, in part by promoting microbial colonization of sclerotia.

### **Nutrients and *in vitro* growth of *Phaeocryptopus gaeumannii*, a fungus causing the Swiss needle cast disease on *Pseudotsuga menziesii***

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The hyphae intercellular development of *Phaeocryptopus gaeumannii*, a foliage pathogen in Douglas-fir trees, is affected by the nutritional composition of the needles. The objective was to examine the effect of different nutrients and silicon, Si, on the *in vitro* growth of the pathogen. Two experiments were established in a completely random design with a fungus isolate (JN204508). The first experiment was by plating the isolate aliquot (1 mm, 0.002 g) in 2% MEA amended with different nutrients, 10 treatments, and incubated at 21°C in darkness for 42 days; diameter colony growth was measured and instantaneous relative rate growth was estimated. The second experiment was in liquid medium, potato extract-dextrose, and amended with nutrients, 21 treatments; incubation was under shaking conditions at 250 rpm at 23°C for 35 days. Mycelium growth was measured by their weight in wet and dry stage. The macronutrients (N P K Ca Mg S) favored the growth of *P. gaeumannii*, in contrast the micronutrients (Fe Cu Zn Mn Mo B) severely inhibited the development of the fungus. The complete nutrient solution plus Si at 1000 ppm reduced colony growth in the agar medium, and at 250 ppm in the liquid medium. Thus, the addition of Si inhibited growth of the fungus and this was attributed to a possible toxic effect of the element. The results provide a basis for designing a fertilization formula for Douglas-fir and allowing to partially inhibit intercellular fungal growth in needles.

### **Adapting to water insecurity: Co-managing oomycete pathogens and water use using deficit irrigation sensor networks**

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Circumglobally, the agricultural sector consumes the highest percentage of available fresh water, and with increases in water insecurity, agricultural demands are becoming unsustainable. Sensor-driven deficit irrigation networks use less water by allowing a mild stress of the crop. The objectives of this study were to evaluate (i) deficit irrigation (DI) as means to reduce root disease in annual and perennial nursery/greenhouse crops; and (ii) effects of DI under various infection scenarios using tomato and *P. capsici* as pathosystem model, to address grower risks hindering technology adoption. In a two year study, Rhododendron were inoculated with *P. cinnamomi* and placed under four different irrigation treatments. Mortality incidence after 18 months of inoculation was significant ( $P = 0.0084$ ), reflecting significantly lower mortality (21%) at 33.5% VWC compared to the 43, and 28% VWC treatments (29, 51% mortality). Preliminary studies on Poinsettia indicate a higher root rot incidence in the 48% VWC deficit irrigation treatment compared to saturated conditions (60% VWC), but differences were not significant. Studies underway in the model pathosystem are evaluating whether DI strategies can predispose plants to pathogen infection, and use of DI as a tool to prevent latent infection and disease. These studies will be used to develop recommendations for using deficient irrigation to co-manage water use, and disease losses in nursery and greenhouse production.

### **Addition of supplemental spent lime to previously limed soils for control of *Aphanomyces* root rot on sugar beet**

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Application of sugar beet factory spent lime to soil in fields infested with the soilborne oomycete pathogen *Aphanomyces cochlioides* has been shown to decrease disease and increase sugar beet yield in Minnesota and North Dakota. Sugar beet growers are inquiring about the need to reapply lime in fields where lime has previously been applied. In a field trial where lime had originally been applied in April 2004 at 0, 6, 12, 18 and 24 Mg dry wt ha<sup>-1</sup>, plots were split and an additional 7.8 Mg dry wt ha<sup>-1</sup> was added to half of each plot on October 31, 2014. The following spring, sugar beet was sown and data was collected on early season stand, *Aphanomyces* root rot and sugar beet yield. There was significant interaction between supplemental and original rates of lime for early season stand, root rot ratings, and yield ( $P \leq 0.05$ ). At 4 and 7 weeks after planting, sugar beet stands were higher in plots that received supplemental lime where original lime rates were 0 and 6 Mg ha<sup>-1</sup> ( $P \leq 0.05$ ), but not where original lime rates were 12 Mg ha<sup>-1</sup> or higher. Supplemental lime reduced *Aphanomyces* root rot and increased sugar beet yield only in plots where lime had not been previously applied ( $P \leq 0.05$ ). Results demonstrate a benefit from adding supplemental lime to fields previously limed at low rates, but do not support a need to add supplemental lime to fields previously limed with at least 12 Mg dry wt ha<sup>-1</sup> within the past ten years.

### **Assessment of genetic diversity and virulence spectrum of *Magnaporthe oryzae* population from sub-Saharan Africa**

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Rice Blast Disease (RBD), caused by the fungus *Magnaporthe oryzae* is a devastating disease found throughout the world and is particularly damaging to rice production in sub-Saharan Africa (SSA). Yield losses of 50-80% are common not only leading to net imports from abroad but also invasion of new pathotypes. An effective strategy to control RBD is to utilize resistant cultivars, but *M. oryzae* evolves rapidly due to genome instability, frequent loss/break of resistance genes occurs, overcoming resistance within few years of deployment. Breeding for durable resistance requires knowledge of pathogen's genetic diversity and virulence spectrum. In our study, genetic diversity of *M. oryzae* field isolates from 9 SSA countries (Benin, Burkina Faso, Ghana, Kenya, Nigeria, Tanzania, Mali, Togo, and Uganda) were assessed by SNPs using GBS technology. Phylogenetic analysis of SNPs from 78 isolates, revealed 7 distinct clades differing in virulence. PCA showed that isolates from East were genetically distinct from West Africa. Further, standard least square regression analysis of virulence spectrum in the 7 clades revealed 4 major groups which differed in disease scores highlighting the association between genetic diversity and virulence. GWAS will be performed to further strengthen the association between genetic diversity and virulence and map the putative avirulence (AVR) genes for functional characterization facilitating an informed deployment of RBD resistance programs.

#### **Quantitative resistance loci identified for the bacterial blight causative agent, *Xanthomonas oryzae* pv. *oryzae***

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Any factor that limits or reduces attainable agricultural yield, including plant disease, will impact global food security. *Xanthomonas oryzae* pv. *oryzae* (*Xoo*) causes the most important bacterial disease of rice, bacterial blight (BB). Deployment of resistance varieties is the gold standard for managing plant diseases, having the strongest impact with minimal environmental effects and cost. However, resistance breeding depends on harnessing genetic diversity in available germplasm for identification of effective and durable disease resistance. To identify durable resistance, we screened 330 advanced inbred lines (AILs) from the *indica* Multi-Parent Advanced Generation Inter-Cross (MAGIC) rice population for resistance to *Xoo* strains, PXO99A and PXO99A harboring TAL-effector TAL7b (+TAL7b). TAL7b, a virulence factor for *Xoo*, allowed us to test the hypothesis that resistance targeted to important virulence factors will be more durable when deployed. Transgressive segregation for resistance was observed to both PXO99A and PXO99A +TAL7b, suggesting the presence of resistance QTL for BB. Genome-wide association (GWA) and interval mapping analysis, revealed 13 disease resistance QTL, six specific to PXO99 +TAL7B and seven to PXO99A. Future exploitation of the MAGIC AILs and screening a new subset of lines selected based on the knowledge acquired from this study will refine the resistance loci, and help elucidate the molecular basis for the resistance phenotypes identified.

#### **Accelerating the pace of developing FHB resistant two-row malting barley: Genomic selection approach**

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Fusarium head blight (FHB) incited by *Fusarium graminearum* is a major disease of barley (*Hordeum vulgare*) in Canada, which significantly impacts seed quality through production of mycotoxins in the grain. While breeding efforts have been ongoing at the Agriculture and Agri-Food, Brandon Research and Development Centre for a couple decades, progress has been limited by requirements for labour-intensive screening nurseries and quantitative based resistance. With advent of affordable genomic tools, breeding for crop characters that are difficult to measure and/or display low heritability are suitable for development through application of genomic selection techniques. A large population of 400 two-row barley lines has been assembled based on diverse reaction to FHB. This collection has been phenotyped in 2014 and 2015 at three locations in Manitoba (Brandon, Carberry, and Carman), and deoxynivalenol content in matured grains was measured by enzyme linked immunosorbent assay (ELISA). This data will be used in combination with genomic information to make predictions of genomic estimated breeding values. Genomic markers will be validated in breeding populations segregating for FHB resistance.

#### **Analysis of Resistance to Blast Isolates and Mapping of Rice Blast Resistance Gene in T2**

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To understand the genetic mechanisms of space-induced rice mutants' resistance to blast, T2, an *indica* rice mutant from TaiHang68, was subjected to resistance spectrum using 39 blast isolates collected from Guangdong Province, China along with main cultivars from Guangdong province. The results showed that T2 conferred broader resistance spectrum than most of main cultivars from Guangdong province. Genetic analysis and gene mapping were also applied in this study. Isolate GD0193 was used for inoculating a population of F<sub>2</sub> derived from the cross between T2 and highly susceptible variety LTH. The results showed that the segregation of resistant (R) and susceptible (S) progenies matched a 3:1 ratio in the F<sub>2</sub> population (1585R:537S,  $\chi^2 = 0.77$ ,  $P > 0.75$ ), indicating that this R gene was controlled by a single dominant gene, temporarily designed as *PiTH* gene. The R gene was mapped between T5 (1.4 cM) and K10 (0.29 cM) at chromosome 11 using SSR and InDel markers, and it was co-segregating with marker K7. *PiTH* may be a novel resistance gene in the *Pik* cluster, which contributes greatly to the blast resistance of T2. Thus, T2 could be used as a new germplasm to facilitate rice blast resistance breeding.

#### **Exploring gene loci controlling bakanae disease resistance by genome-wide association mapping**

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Bakanae disease, caused by *Fusarium fujikuroi*, is a rice seed-borne disease and widely distributed in rice growing areas. Infected rice seedlings may show complex symptoms including abnormal elongation of stem or internodes, wider leaf angle, slenderness, and even die, causing considerable yield and quality loss. In recent years, studies have reported the rising incidence and severity of the disease in Asia. Even though the disease has been known over a century, little is known about the resistance genetics and resources in rice. In this study, we used genome-wide association mapping approach to identify gene loci controlling bakanae disease resistance. A set of 413 diverse rice varieties from 82 countries (rice diversity panel; containing 44,100 SNPs available from open-source) was artificially inoculated and disease levels were evaluated by visual observation using a 0-3 rating scale, detailed image analysis using a newly developed MATLAB-based software and quantification of *F. fujikuroi* colonization in rice seedlings with a selective medium. A total of 24 QTLs associated with disease severity and/or pathogen colonization were mapped by the mixed linear model in TASSEL software. Defense- and hormone-related candidate genes identified within the QTLs will be further validated.

#### **QTL Mapping of Resistance to Tan Spot in a Winter Recombinant Inbred Line Population Derived from Cross between Harry and Wesley**

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Tan spot, caused by the *Pyrenophora tritici-repentis*, is an important disease on both common and durum wheat worldwide. The disease system is known to involve three necrotrophic effectors (NEs) that interact with corresponding host sensitivity genes, including Ptr ToxA-*Tsn1*, Ptr ToxB-*Tsc2* and Ptr ToxC-*Tsc1*. To investigate the role of NE-host sensitivity interactions in disease, we conducted the QTL mapping of tan spot resistance in a recombinant inbred line population derived from a cross between two US winter wheat cultivars 'Harry' and 'Wesley'. Harry was sensitive to Ptr ToxA and highly susceptible to tan spot while Wesley was insensitive and highly resistant. QTL mapping revealed two major loci: the one corresponding to the *Tsn1* locus, and one on 1AS, which likely corresponded to the *Tsc1* locus. Both the *tsn1* and *tsc1* loci were significantly associated with resistance to race 1, but only *tsn1* was significantly associated with race 2, and only *tsc1* was associated with race 3. Neither locus was significantly associated with disease caused by a race 2 *ToxA* knockout strain. Additional QTL were also identified in this population, but their effects on disease were relatively minor. This work indicates that the Ptr ToxA-*Tsn1* and Ptr ToxC-*Tsc1* interactions play important roles in conferring tan spot susceptibility in winter wheat, and breeding programs should remove these susceptibility loci in order to improve tan spot resistance.

#### **Molecular mapping of effective stripe rust resistance genes in wheat germplasm PI 182126**

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Stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most damaging diseases of wheat. Spring wheat landrace PI 182126, originally from Sind, Pakistan, has shown a high level of resistance to stripe rust in field experiments for more than 10 years and in greenhouse tests with several predominant races. The objective of this study was to map the resistance gene(s) in PI 182126. A population of 120 F<sub>3</sub> recombinant inbred lines was developed from the cross between PI 182126 and susceptible genotype Avocet S through single-seed decent. The population was tested with US predominant races PSTv-14 and PSTv-37 at seedling stage under greenhouse conditions and at adult-plant stage in fields under natural infection of the pathogen near Pullman and Mount Vernon, Washington in 2014. The population was genotyped using microsatellite markers. Two resistance genes were mapped to chromosomes 5B and 7B. Using Chinese Spring nulli-tetrasomic and 7B deletion lines, the gene on 7B was mapped to the 0.14 bin of the long arm. Based on the chromosomal location, tightly linked markers, and effective throughout growth stages against all races tested, the gene on 7B is different from *Yr39*, *Yr52*, *Yr59*, *YrZH84*, and *YrC591* previously mapped to the long arm of chromosome 7B, and is useful for diversifying stripe rust resistance genes used in breeding programs.

#### **SNP variation in switchgrass disease resistance genes**

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Switchgrass (*Panicum virgatum* L.), an allotetraploid perennial C4 grass native to North America, is an important feedstock for cellulosic biofuel production. Its high genotypic and phenotypic variation yield substantial potential for genetic improvement of bioenergy-related traits, including disease resistance. We studied SNP variation in 276 switchgrass genotypes, belonging to 36 accessions from mainly the Southern USA, across ~1 kb NBS-LRR regions of 4 classes of putative resistance (R) genes. PCR amplicons for an R gene with homology to the barley powdery mildew resistance gene *Mla* from 150 switchgrass accessions mapped to two homoeologous regions in the allotetraploid switchgrass genome. Reads from individual accessions aligned preferentially against only one locus. Fifty-eight SNP positions and 78 haplotypes were recorded. Twelve SNPs were present in more than 35% of the accessions. Although switchgrass is largely an obligate outcrossing species, 95% of the SNPs were homozygous. The results indicate that selection for some potentially beneficial SNPs occurred in natural switchgrass population which were subsequently fixed in homozygous condition at the locus level.

#### **Development of molecular makers for spinach resistance genes to downy mildew disease**

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Spinach is an increasingly popular vegetable crop. Downy mildew, caused by *Peronospora farinosa* f. sp. *spinaciae* (Pfs), is the most economically important disease of spinach and the main focus of all spinach breeding programs. Resistant cultivars are the most economical way to manage downy mildew, particularly for organic spinach production, which is approaching 50% in the US. Resistance to downy mildew is typically controlled by single dominant genes, and 11 resistant loci have been hypothesized in spinach. Three resistant loci, RPF1, 2 and 3 have been genetically characterized; each provides resistance to more than 10 of the 16 described races of Pfs. A molecular SCAR marker, linked to RPF1, has been shown to be useful in selecting this locus for resistance. Bulk segregant analysis and genotyping by sequencing approaches were employed to develop markers for RPF2 and 3. One out of 900 10-mer random primers examined generated a fragment specific to resistant plants, and the derived SCAR marker co-segregated with the RPF2 locus. For RPF3, a SNP linkage map was constructed with two SNP markers closely linked (1.1 and 4 cM) to the RPF3 locus. The RPF2 and RPF3 markers have been shown to be effective in selecting the respective resistance loci outside of the original mapping populations demonstrating their utility. These markers could be used to pyramid resistant loci to improve the durability of downy mildew resistance in spinach.

#### **Genome-wide association study of transmission of Soybean mosaic virus through seed**

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Soybean mosaic virus (SMV), an aphid- and seed-transmitted member of the *Potyviridae*, causes significant reductions in soybean yield and seed quality worldwide. In North America, seedborne infections serve as the primary sources of inoculum for SMV infections. Therefore, host-plant resistance to seed transmission of SMV provides a means to limit the impact of SMV on soybean production. In this study, populations of 200 and 477 plant introductions from the United States Department of Agriculture Soybean Germplasm Collection were evaluated for their transmission of SMV through seed. To identify loci associated with resistance to SMV seed transmission, the phenotypic data and single nucleotide polymorphism (SNP) information for each line from the SoySNP50K resource were combined and analyzed using a compressed mixed linear model as implemented in the GAPIT package in R. The loci identified were compared to the results from previous biparental mapping studies of resistance to SMV seed transmission. This study provides a comprehensive analysis of loci associated with seed transmission of an economically important plant virus.

#### **Identification of resistance genes and resistance gene analogs by si-RNA sequencing of the sweet potato leaf phytobiome**

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Sweet potato (*Ipomoea batatas* L. (Lam)) is affected by single and synergistic virus co-infections manifested as biotic stress and severe disease. Consequently, breeding for disease resistance is used in disease management. However, there are few reports of resistance genes (*R*-genes) against specific viruses in sweet potato virus disease complex (SPVD). This study sought to identify *R*-genes in the sweet potato leaf phytobiome using Next Gen-sequencing. RNA-seq has been used to characterize viruses causing SPVD while discovery of *R*-genes traditionally involves cloning and characterization of resistance proteins and resistance gene analogs (RGA). In this study, RNA was extracted from three sweet potato cultivars (CBS 49, CBS 32 and C-104) symptomatic with *Sweet potato feathery mottle virus* (SPFMV), *Sweet potato leaf curl virus* (SPLCV) and Sweet potato badnavirus A and B. These were then subjected to RNA-seq analysis and bioinformatics analysis of the assembled contigs for resistance genes. We report the presence of transcripts of a total of thirty-one such genes, and putative proteins representing several classes of resistance genes including nucleotide-binding site leucine-rich repeat (NBS-LRR) and RGA from sweet potato such as AB125881, DQ903328, and DQ903597 in the sweet potato phytobiome. These results provide a genome resource of *R*-genes for development of tagged molecular markers useful for mapping disease resistance in specific sweet potato cultivars.

#### Differential effects of stalk rot diseases on photosystem II photochemical efficiency among resistant and susceptible sorghum lines

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Stalk rots are among the most injurious sorghum diseases worldwide. Understanding the plant physiological changes that occur during these diseases will help to uncover potential resistance mechanisms. The objective of this study was to test the effect of stalk rots on sorghum (*Sorghum bicolor* (L.) Moench) leaf chlorophyll content and photosystem II photochemical efficiency (Fv/Fm). Two resistant (SC599, SC35) and susceptible (Tx7000, BTx3042) lines were field evaluated in 2014 and 2015. Plants were inoculated with *Fusarium thapsinum* (Fusarium stalk rot), *Macrophomina phaseolina* (charcoal rot), and phosphate-buffered saline (control) at 14 d after flowering. Flag leaf chlorophyll and Fv/Fm were measured at soft-dough, hard-dough, and physiological maturity using a Soil and Plant Analytical Development meter (SPAD) and a chlorophyll fluorometer, respectively. ANOVA was performed with appropriate modeling for repeated measures. Compared to control, both pathogens significantly reduced SPAD ( $P < 0.0001$ ) across genotypes and growth stages and the reduction was greater in 2014. Across years and growth stages, both pathogens significantly reduced the Fv/Fm ( $P < 0.016$ ) of susceptible lines while no such effect was evident for the resistant lines. Despite the apparent chlorophyll reduction during disease development, the ability to maintain higher photosynthetic activity at the tested growth stages appeared to contribute to physiological resistance against stalk rot diseases.

#### Two SREBP Transcription Factors are Required for Ergosterol Biosynthesis and DMI Resistance in *Penicillium digitatum*

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Application of imazalil is a common method for control of citrus green mold caused by *Penicillium digitatum*. As a consequence of continuous use, imazalil-resistant *P. digitatum* strains have emerged worldwide. Our previous study showed that overexpression of imazalil target genes CYP51A and CYP51B, induced by the insertion mutations in their promoter regions, is responsible for the resistance. However, the regulators inducing overexpression of CYP51s are unclear. In this study, we demonstrated two SREBP homologs, designated as PdSrbA and PdSrbB. Both ?PdSrbA and ?PdSrbB showed markedly reduced ergosterol contents and increased sensitivities to imazalil and other DMIs, while double deletion led to hypersensitive to DMIs. ?PdSrbA but not ?PdSrbB is hypersensitive to terbina?ne and tridemorph, two SBIs targeting to ERG1 and ERG24, respectively. In addition, PdSrbA also mediated responses to iron-depletion and hypoxia. However, no change was observed in growth rate or virulence for those mutants. Expression of CYP51s and other genes involved in ergosterol biosynthesis was significantly down-regulated in ?PdSrbA and ?PdSrbB. These results suggested that these two SREBP homologs co-regulate ergosterol biosynthesis as well as DMIs resistance in *P. digitatum*, and PdSrbA may be with wider functions.

#### Chemical association of potato susceptibility to *Rhizoctonia solani*

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*Rhizoctonia solani* is the causal agent of stem canker and black scurf of potato. To investigate chemical factors related to potato resistance to *R. solani*, 17 cultivars of potato were selected for a two-year field trial. Sixty-day-old plants were dug from field, and wax content of underground stems was measured. For other chemical measurement, potato seedlings grown in Murashige and Skoog medium to an approximate height of 12 cm, were transferred into greenhouse pots containing vermiculite and soil (v:v = 1:1). After 30 days, plants were dug and the underground stems were used to determine the silicon content. Leaves were used to determine the content of soluble sugar, soluble starch, free amino acid and soluble protein. Black scurf was measured based on 0 to 5 scale, which was used for disease index calculation. Wax content of underground stems of field-grown potato was negatively correlated with disease index ( $r = -0.532$   $P < 0.05$ ), but silicon content did not have a significant correlation ( $r = 0.042$   $P > 0.05$ ), indicating wax-enhanced potato resistance to *R. solani*. The correlation between soluble starch, soluble protein, soluble sugar and free amino acid and disease index were 0.476 ( $P < 0.05$ ), 0.458 ( $P < 0.05$ ), 0.300 ( $P > 0.05$ ) and 0.219 ( $P > 0.05$ ), respectively. This showed that lower levels of soluble starch and higher levels of soluble protein enhanced disease resistance, while soluble sugar and free amino acid had no significant effects on disease resistance.

#### Genetic characterization of quantitative resistance to *Bremia lactucae*, the causal organism of lettuce downy mildew

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Lettuce (*Lactuca sativa*) is one of the most valuable vegetable crops in the United States. Downy mildew (DM), caused by *Bremia lactucae*, is the most important foliar disease of lettuce worldwide, which decreases the quality of the marketable portion of the crop. The use of resistant varieties carrying dominant genes (*Dm* genes) is the most effective method for controlling this disease. However, the high pathogen variability leads to the defeat of cultivars containing resistance genes by new isolates of the pathogen. Some lettuce varieties such as Iceberg and Grand Rapids exhibit field resistance that cannot be attributed to *Dm* genes. This resistance trait, which is manifested in adult stages of development, has shown to be quantitatively inherited. To determine the genetic basis of field resistance, two populations of recombinant inbred lines originated from crosses between a field resistant cultivar and a susceptible cultivar (Grand Rapids x Salinas and Iceberg x PI491224) have been evaluated for DM severity and genotyped using Genotyping-By-Sequencing for quantitative trait loci (QTL) analysis. Marker-assisted gene pyramiding of multiple *Dm* genes in combination with QTLs for field resistance provides the opportunity for more durable resistance to *B. lactucae*.

#### *Exserohilum turcicum* races causing northern leaf blight of corn in the North Central United States

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Northern leaf blight of corn, caused by *Exserohilum turcicum*, is a yield-reducing foliar disease that commonly occurs across the North Central U.S. Previous race population distribution studies identified five physiological races in the U.S. prior to 1995. For this study, 156 *E. turcicum* isolates from Illinois, Indiana, Iowa, Minnesota, North Carolina, Ohio, and Wisconsin were screened against corn differential lines containing *Ht1*, *Ht2*, *Ht3*, *Htm1*,

and *Htm1* resistance genes. Isolates were collected between 1979 and 1985 (n=13) and between 2007 and 2014 (n=143). Twenty physiological races were observed based on the responses of the differential corn lines. Races 0, 1, and 1mn were the most prevalent races, comprising 21%, 27%, and 13% of the isolates, respectively. Races were diverse within all states and years. Virulence to multiple *Ht* resistance genes within individual isolates was observed in 47% of those tested, with 3% of the isolates conferring virulence to all *Ht* resistance genes tested. Virulence to the *Ht1*, *Ht2*, *Ht3*, *Htm1*, and *Htm1* resistance genes was present in 64%, 20%, 18%, 32%, and 27% of the *E. turcicum* isolates, respectively. Virulence to *Ht* resistance genes was fairly evenly distributed across states in isolates collected after 2008. Although *Ht* genes were still effective against several isolates, the use of quantitative resistance in conjunction with *Ht* genes would likely improve management of northern leaf blight in the North Central U.S.

#### **Wheat yellow rust dynamics in Tunisia since 2013 and resistance genes in durum wheat**

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Wheat yellow rust epidemics, caused by *Puccinia striiformis* f. sp. *tritici* (PST) have increased worldwide with the emergence in Western Europe and North Africa of an aggressive strain and tolerant to high temperature (PstS2) since 2004 and exotic strains (Triticale 2006, 2015, Warrior and Kranich) recently. Even though resistant varieties bring to agriculture effective solutions to reduce the use of pesticides, races of the pathogen quickly overcome introduced resistance genes. In this study we determined the virulence combinations using the European and world differential sets and the simple sequence repeat diversity of 94 PST isolates collected in Tunisia since 2013 from the major wheat growing areas. In addition, 39 local Tunisian durum wheat accessions and 109 recombinant inbred lines RILs (F9) were evaluated at the seedling stage against old and new races of PST in order to identify resistant genotypes and markers associated with resistance genes. The results underlined the rapid change in wheat yellow rust population where 3 predominant races, Warrior1 (W1), Warrior- (W-) and Triticale 2006 were detected since 2013. In addition, 10% of the durum wheat accessions were resistant to W1, W- and PstS2. The evaluation of the RILs issued from a cross between Tunisian durum wheat Khair and Agili39 revealed one major resistance gene against W1 and two minor genes against PstS2. The use of these genes in breeding can improve durability of the genetic control against PST.

#### **Effectiveness of adult plant resistance to wheat stem rust is specific to *Puccinia graminis* f. sp. *tritici* race**

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Adult plant resistance (APR) in wheat to stem rust caused by *Puccinia graminis* f. sp. *tritici* is considered to be conferred by multiple loci that are race non-specific. Our objective was to test the race-specificity of individual and combinations of wheat stem rust APR loci. We evaluated a biparental wheat population (Thatcher/McNeal) that is segregating for six APR loci with minor effects in response to four races of the stem rust pathogen (QTHJC, TPMKC, RCRSC, and QFCSC), in single-race field nurseries over three years. QTL mapping and ANOVA identified the significance and effectiveness of the six minor effect loci across the years and races. The QTL with the largest effect, coincident with the *Sr12* locus, was effective to virulent races QTHJC and TPMKC, but conferred a variable, sometimes nonsignificant, response to race RCRSC. The other five QTL varied in effectiveness between years and were generally less effective in response to race RCRSC in particular. The data from QTL mapping suggested that some of the QTL could be race specific, such as *QSr.cdl.1AL*, because they were detected in response to races such as TPMKC and QTHJC, but not to race RCRSC. This observation has implications specifically for wheat stem rust resistance breeding because it suggests that resistance observations to a single race may not be indicative of resistance to another race, even if the mechanism of resistance is multiple minor effect loci.

#### **Lack of interaction between *Fusarium oxysporum* f. sp. *niveum* and *Meloidogyne incognita* on cucurbit rootstocks resistant to *Fusarium* wilt of watermelon**

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Interspecific hybrid squash (*Cucurbita maxima* × *C. moschata* ‘Strong Tosa’) and bottle gourd (*Lagenaria siceraria* ‘Macis’) rootstocks are resistant to *Fusarium oxysporum* f. sp. *niveum* (FON) but susceptible to *Meloidogyne incognita*, Southern root-knot nematode. Co-infection with root-knot nematode has rendered *Fusarium* resistance ineffective in other hosts. The objectives of this study were to determine if such an interaction occurred between *M. incognita* and FON races 1 and 2 on ‘Strong Tosa,’ ‘Macis,’ and watermelon ‘Fascination’ (resistant to race 1) and ‘Tri-X 313’ (susceptible to both races). The four hosts were inoculated in a greenhouse with one of four pathogen treatments: FON applied as  $2 \times 10^7$  microconidia per pot, *M. incognita* applied as 2000 J2 per pot, both pathogens, or neither pathogen. Each race was tested twice with 10 replicate plants per host and inoculation treatment. Plants not inoculated with FON did not wilt. After 4 weeks, severity of *Fusarium* wilt and recovery of *F. oxysporum* did not differ when hosts were inoculated with FON alone or with FON and *M. incognita* together (host-by-treatment interactions not significant,  $P \geq 0.08$  and  $P \geq 0.17$  for races 1 and 2, respectively). Galling was present on  $\geq 10\%$  of the root system of 90% of the plants inoculated with *M. incognita*, and no non-inoculated plants had galls. In conclusion, resistance to FON in cucurbit rootstocks is not compromised when *M. incognita* infects the roots.

#### **Adaptation to quantitative resistance in the hop cultivar Cascade by *Podosphaera macularis***

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In the hop powdery mildew pathosystem, the cultivar Cascade has not been substantially affected by powdery mildew despite being produced on a relatively broad scale for decades. Beginning in 2012, development of powdery mildew on this cultivar under field conditions has become increasingly common in Washington State. Surveys conducted during 2013 to 2015 indicated increasing occurrence and severity of powdery mildew on Cascade, concomitant with increases in the number of fungicide applications made growers. Nearly all isolates of *Podosphaera macularis* sporulated when inoculated to Cascade, although the latent period was shortest, infection frequency greatest, and the fungus most fecund on Cascade when inoculum was originally derived from this cultivar as compared to other cultivars. Only 4.9% of isolates obtained from Cascade were able to infect cv. Nugget, which possess the resistance gene termed R6, indicating that Cascade-adapted strains of the fungus are distinct from strains that attack cultivars possessing R6. In growth chamber experiments, powdery mildew levels on various cultivars were similar when inoculated with Cascade-adapted strains of *P. macularis* as compared to non-adapted strains, further supporting a specific adaptation by the fungus to the quantitative resistance in Cascade. Multiple R-genes are predicted to provide resistance to Cascade-adapted strains of the fungus, as is quantitative resistance found in other germplasm.

#### **Gene-For-Gene in Fusiform Rust Disease of Loblolly Pine**

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The rust fungus *Cronartium quercuum* f.sp. *fusiforme* (*Cqf*) incites fusiform rust disease on southern pines causing loss of stem quality or death. Given that fusiform rust is a coevolved, gene-for-gene pathosystem there is potential for resistance to be overcome in the field. Nine resistance genes (*Fr1-Fr9*) and one avirulence gene (*Avr1*) have been placed on the genetic maps of loblolly pine and *Cqf*. More recently genomic references have become available for both the host and the fungus. Allelic-level sequence data for resistance genes and their corresponding avirulence genes would make it possible to monitor virulence in the field, guide deployment and understand local outbreaks. Toward that end work is underway in both the loblolly host and *Cqf* pathogen. A candidate for the *Fr1* resistance gene was identified in the 22 gigabase pair, loblolly pine reference genome. This gene remains a candidate

and fine mapping is currently being done to improve the loblolly genetic map in this region, as well as, identify additional resistance gene candidates from elite pine families. Research on the *Cgff* fungus has located a genomic interval that contains the *Avr1* gene using both traditional marker sequences and high-throughput re-sequencing of progeny from of an *Avr1* segregating cross. Using an improved *Cgff* assembly and re-sequencing of field isolates, we are attempting define the *Avr1* gene and the causative mutation that led to virulence in the *Avr1* mapping population.

#### **Melampsora rust running amuck: Exploring willow rust population diversity in the Northeast United States**

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Shrub Willow (*Salix* spp.) has proved to be a reliable short-rotation coppice (SRC) biofuel feedstock in the Northeast United States. However, the willow rust pathogen *Melampsora* spp. can cause up to a 50% reduction in yield, making some growing operations economically limited. This has spurred investigation into both the identification of resistance within in *Salix* spp. and the diversity of *Melampsora* spp. in the Northeast. Approximately 200 single pustule isolates were collected from MI, NY, PA, VT, and WV, and are being characterized by rDNA sequencing. Preliminary data shows that both *M. americana* and *M. paradoxa* are present in our regions of interest. Further investigation of *Melampsora* spp. diversity throughout the Northeast will be conducted using genotyping-by-sequencing, a genome wide high-throughput SNP identification method, providing thousands of markers for population studies targeting un-methylated coding sequences. Additionally, we explored disease resistance by characterizing association and linkage mapping populations of *Salix purpurea* with the aim of identifying QTL and tightly-linked SNPs for marker-assisted selection. Preliminary data show a continuum of resistance, suggesting promising breeding opportunities. This information will lead to further understanding of the population biology of *Melampsora* willow rust, while providing crucial information necessary for the success of future willow breeders.

#### **Marker development and fine-mapping for the Phytophthora crown rot resistance locus, *Pc1*, in strawberry**

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Phytophthora crown rot (PhCR) caused mainly by *Phytophthora cactorum* is a destructive disease and can cause economic losses to strawberry growers in US. In our previous study, a major QTL for PhCR resistance, *Pc1*, located on the linkage group 7D, was discovered in complex and multi-family populations using the whole-genome SNP genotyping (IStrow90 Axiom<sup>®</sup> SNP Array) and pedigree-based marker-trait association analysis (FlexQTL<sup>TM</sup>). For the fine-mapping of *Pc1*, a total of 25 SNP-based high resolution melting (HRM) markers and 63 simple sequence repeat (SSR) markers were selected in the *Pc1* region. All markers were tested with five strawberry varieties and 20 advanced breeding selections that inherited putative functional allele combinations of *Pc1pc1* (heterozygous resistant) or *pc1pc1* (susceptible). Total five markers were found to co-segregate with the resistance locus, *Pc1*. In addition, we developed three populations (n=339) by crossing the resistance parent (*Pc1pc1*), '12.55-220', with three susceptible accessions (*pc1pc1*), '12.82-44', '11.116-56', and '11.98-41'. All populations were planted to the field (Wimauma, FL) and evaluated for the PhCR disease resistance. The HRM and SSR markers linked to the *Pc1* resistance locus were tested for the PhCR fine-mapping population, and the linkage analysis was conducted. The precise location of *Pc1* and DNA markers developed in this study will be utilized for the PhCR resistance breeding program.

#### **Mitigating the effects of shoot blight caused by *Erwinia amylovora* by enhancing plant systemic resistance**

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Fire blight, caused by *Erwinia amylovora*, is one of the most devastating diseases of apple and pear and continues to cause large economic losses for tree fruit producers. Antibiotics are largely used to control blossom blight where bacterial resistance is absent, but the need exists for alternative control strategies where resistance occurs, and where shoot and canker blight are prevalent. One strategy is through controlled activation of the plant immune system by the application of materials containing plant defense activating compounds. Our goal was to test the effectiveness of an experimental resistance activating compound in reducing shoot blight severity. We applied three different concentrations of the chemical as foliar sprays on potted 'Gala' apple trees grafted on M.26 rootstocks. Three concentrations were applied at three, two, and one week before shoot tip inoculation and three, two and one day before shoot tip inoculation; Acibenzolar-S-methyl (Actigard) was also applied at three, two, and one week before shoot tip inoculation. Evaluations of shoot severity were made at one and two weeks after inoculation. Treatments started three days before inoculation reduced severity by 9, 26, and 35% for the three concentrations tested. Treatments started three weeks before inoculation reduced severity by 27, 21, and 44%, for the concentrations tested. Acibenzolar-S-methyl applications reduced severity by 75%. The testing of other alternatives is ongoing.

#### **Induction of resistance in cultivated peanut against peanut early leaf spot by salicylic acid**

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Cultivated peanut (*Arachis hypogaea* L.) is an economically important crop for the United States and throughout the world. *Cercospora arachidicola* (Ca), the causal agent of early leaf spot (ELS) on cultivated peanut, can cause yield losses of up to 50%. Since earlier work reported inconsistent results with salicylic acid (SA) relative to peanut pathogens, we sought to examine the ability of SA to induce resistance against ELS on a high-oleic cultivar. We evaluated the effect of spraying three rates of SA (100, 200, and 300  $\mu$ M) at 3, 5, and 7 days before inoculation (DBI) with ELS in whole peanut plants (cultivar GA-09B) in a growth chamber. Each treatment was replicated three times. At 15 days after inoculation (DAI), all SA treatments had significantly fewer lesions (3.1, 2.8, and 2.5 lesions with increasing SA rates; and 1.9, 3.0, and 3.6 lesions with increasing DBI), compared with the control (12.7). At 42 DAI, all sporulating lesions from SA treated plants tended to produce fewer spores compared with the control (170.0); for example, 27.2 with 300  $\mu$ M SA, 54.5 with 3 DBI, and 31.0 with 7 DBI. Although there were fewer lesions on SA treated plants, these lesions were larger than those on the control. Results of this study suggest that SA plays at least a transient role in ELS resistance in peanut cultivar GA-09B.

#### **Development of a molecular marker platform for the breeding of blast resistance varieties in Taiwan**

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Rice blast is the most important disease commonly occurring in the first cropping season in Taiwan. To facilitate marker-assisted backcrossing, this study aims to identify effective blast resistance (*R*) genes in Taiwan and develop *R* gene and background selection markers for the use of IRR1-bred blast resistant lines (IRBLs) as donors. The resistance spectra of known *R* genes were evaluated by inoculating 31 IRBLs with 18 *M. oryzae* isolates collected from six rice-growing areas in Taiwan in 2009-2013. The *R* alleles at *Pi2/9*, *Pik*, and *Pita/Pita2* were resistant to more isolates. Genotyping-by-sequencing technology was used to analyze 13 IRBLs with broader-spectrum resistance and 20 Taiwan high-quality rice varieties. Based on the 131,942 SNPs between IRBLs and Taiwan varieties, a set of 11 polymorphic *R* gene markers and 96 Fluidigm markers evenly distributed on rice genome were developed. Two *M. oryzae* isolates 12YL-TT4-1 and 13TN-HB1-3 were used to determine which Taiwan varieties would be suitable recipients for the 13 *R* alleles. The result showed that Taikeng 4, Taikeng 9, Taikeng 10, Taikeng 14, Taikeng 15, Taikeng 71, Taiyuan 3, Tainan 11, Kaohsiung 145, and Kaohsiung 147 do not carry the *R* alleles in IRBL9-W, IRBLZ5-CA, IRBLZ5-CA (R), IRBLZ-Fu, IRBL1-CL, IRBL7-M, IRBLK-Ka, IRBLKp-K60, IRBLKm-Ts, IRBLkh-K3, IRBL20-IR24, IRBLta2-Pi, and IRBLta2-Re. The 13 IRBLs and newly developed markers can be used to improve blast resistance of the 10 high-quality rice varieties.

### **Introgression of crown rot resistance into cultivated squash *Cucurbita moschata*, *C. maxima* and *C. agrosperma***

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*Phytophthora capsici* incites crown rot in squash and often causes significant losses to growers worldwide. No resistance is available in commercial squash varieties, but resistance has been found in the wild species *Cucurbita lundelliana* and introduced into *C. moschata*, resulting in several resistant lines. However, the yields of these resistant lines are too poor to warrant their commercial release. To overcome this, four of these resistant lines were crossed with commercial varieties of *C. moschata*, *C. maxima* and *C. agrosperma* in single or three-way crosses. The F<sub>1</sub> progeny of *C. moschata* 'Butterbush' and *C. moschata* resistant lines were resistant to *P. capsici* and showed improved yield. Interspecific cross of 'Buttercup' (*C. maxima*) x 399 (432) (a resistant line) was made. The F<sub>1</sub> progeny were completely resistant to *P. capsici*, and showed strong heterosis in plant growth and fruit size, but were male sterile. Backcross progeny exhibited low seed settings. Three-way crosses of *C. agrosperma* x F<sub>1</sub> (*C. moschata* x *C. moschata*) were obtained. The hybrids showed improved seed settings with higher percentage of normal seeds (41.2%) compared to the F<sub>1</sub> interspecific progeny of *C. maxima* x *C. moschata* (8.8%). Embryo rescue was employed to overcome interspecific barriers. Further backcrossing with recurrent parents and pedigree selection for *P. capsici* resistance, high fruit yield, and flesh quality will be performed.

### **Evaluating soybean breeding lines developed from different sources of resistance to Phomopsis seed decay**

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Phomopsis seed decay (PSD) causes poor soybean seed quality worldwide. The primary causal agent of PSD is *Phomopsis longicolla* (syn. *Diaporthe longicolla*). Breeding for PSD-resistance is the most effective long-term strategy to control this disease. To develop soybean lines with resistance to PSD, multiple breeding populations were developed from crosses between PSD-resistant and high-germination lines, as well as between PSD-resistant lines. Based on the seed plating assay, the range of percent seed infected by *P. longicolla* among all lines tested was from 0 to 52%, with an overall line mean of 7.5. There were significant differences in the reaction to PSD among lines derived from the same source of resistance, as well as among lines from different sources of resistance. More important for making future populations, the mean PSD score of the lines derived from the PI 424324B source was significantly lower than that of those derived from the PI 80837/SS93-6181 source. The PSD of lines from PI 417050 source were not significantly different from those from the other sources. These results indicate an apparent superiority for using the PI 424324B source of resistance for breeding improved lines. Seeds for many of the lines derived from both PI 424324B and PI 417050 had no incidence of PSD. Therefore, it is likely that some of them will be released in the future as resistant to PSD and thereafter utilized in breeding programs to develop PSD-resistant cultivars.

### **Transgenic expression of pattern recognition receptor *EFR* in tomato leads to effective field resistance to bacterial wilt**

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Bacterial wilt of tomato caused by *Ralstonia solanacearum* is a major problem in tomato production in the tropics and sub-tropics worldwide and can cause 50-80% yield losses. We investigated whether transgenic tomato (*Solanum lycopersicum*) expressing the resistance gene *EFR* (Elongation Factor Tu-Receptor) from *Arabidopsis thaliana* or *Bs2* gene from *Capsicum annuum* or both provides improved resistance to bacterial wilt disease in a field with *R. solanacearum*. The presence of *EFR* and *Bs2* or *EFR* alone on the highly susceptible FL 8000 background, significantly reduced the bacterial wilt incidence compared to *Bs2* line (carrying only *Bs2*) or non-transformed control. Although yields of marketable fruit from *EFR* or *EFR/Bs2* lines were statistically not different than those from *Bs2* lines or non-transformed control, the total yield was significantly improved in the *EFR* and *EFR/Bs2* lines. Following harvest, the basal and middle stem sections of the remaining living plants in the field were assessed by diluting plating on modified SMSA media. The results revealed no significant differences in the populations at the basal stem. Interestingly, in the middle stem, no bacteria could be recovered from *EFR* lines or *EFR/Bs2* lines but *R. solanacearum* populations were recovered from *Bs2* and non-transformed control lines at 10<sup>4</sup> CFU/g and 10<sup>3</sup> CFU/g tissue, respectively. This finding indicates the potential of *EFR* gene for field management of tomato bacterial wilt.

### **Plant defensins inhibit growth of pathogens in the alfalfa crown rot disease complex**

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Alfalfa crown rot is a disease complex that severely limits alfalfa stand density and productivity in all alfalfa-producing areas. Currently, there are no viable methods of control. Plant defensins are small cationic antimicrobial peptides with a conserved signature of cysteines. Defensins have a gamma-core motif, a cluster of positively charged residues, which is essential for antimicrobial activity. The core motifs of five synthetic defensins were tested for antimicrobial activity against the pathogens in the alfalfa crown rot disease complex. In a 96-well microplate, each well contained half strength potato dextrose broth, approximately 2000 spores, and concentrations of defensin peptide up to 30 ug/mL in a total volume of 100 uL. After 48 h of incubation at 25°C in the dark, absorbance of the wells was measured at 595 nm on a microplate reader to quantify the inhibition of fungal growth. The amount of defensin needed to inhibit growth of pathogen strains by 50% (EC<sub>50</sub>) was calculated. The core motif of MtDef4 was shown to be the most effective peptide with EC<sub>50</sub> values of 5.3 uM against *Phoma medicaginis* and 6.9 uM against *Fusarium oxysporum* f.sp. *medicaginis*. In addition, MtDef4 had activity against *Pseudomonas syringae* pv. *syringae* and *Xanthomonas alfalfa* but not the oomycete *Aphanomyces euteiches* in *in vitro* assays. These results indicate that transgenic expression of plant defensins in alfalfa has the potential to lead to improved crown rot resistance.

### **A novel vector system to engineer begomovirus resistance in transgenic plants by transcriptional and post-transcriptional gene-silencing mechanisms**

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To generate transgenic plants that are highly resistant to whitefly-transmitted *Tomato leaf curl Taiwan virus* (ToLCTWV) and *Tomato yellow leaf curl Thailand virus* (TYLCTHV), plasmid vectors were constructed to induce transcriptional gene silencing (TGS) and/or post-transcriptional gene silencing (PTGS) in a transgenic plant. The intergenic region (IR) of begomovirus containing inverted repeat sequences was inserted into the intron of a tomato gene encoding a RNA-directed RNA polymerase 1 (RDR1) or a proline dehydrogenase (PDH). Expression of a GFP-RDR in construct carrying a TYLCTHV IR (IRhp), tomato intron and GFP led to nuclear localization of IRhp RNAs in plant cells after intron was spliced out and induced TGS. A C1C2C3 construct carrying the 3' end of C1 ORF and the overlapping region of C2 and C3 ORFs of TYLCTHV triggered PTGS in the cytoplasm. Infection assays revealed that constructs inducing TGS or PTGS alone displayed moderate resistance to TYLCTHV. However, C-RDRin-IR constructs carrying an IRhp, intron and C1C2C3 ORFs induced both TGS and PTGS and displayed strong resistance to TYLCTHV. Constructs carrying RDR1 introns could be easily spliced out and triggered TGS after transient expression in tobacco plants. Constructs carrying PDH intron3 had lower efficiencies in splicing and in triggering TGS. Our results indicated that a combination of inducing both TGS and PTGS in a construct shows great promise in developing resistance against plant viruses.

### Exploiting the approaches of biotechnology in sweetpotato for virus diseases resistance

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Sweetpotato (*Ipomoea batatas* (L.) Lam.; *Convolvulaceae*), a plant widely grown in all tropical and subtropical areas, is among the 10 most important food crops worldwide. As a crop produced by vegetative propagation, “cultivar decline” due to viral infections significantly reduces sweetpotato yield and storage root quality. In the United States, *Sweet potato feathery mottle virus* (SPFMV) and *Sweet potato leaf curl virus* (SPLCV) are two of the most prevalent sweetpotato viruses that cause devastating diseases and yield reductions. Even though resistance to virus infections remains the most cost effective way of disease management, to date, immune resistance to these viruses has not been identified in *I. batatas*. In this study, we explored a novel biotechnological method to develop transgenic sweetpotato plants with resistance to these two viruses. We engineered part of coat protein (CP) gene of SPFMV and the replication origin region of SPLCV into a binary vector for *Agrobacterium*-mediated genetic transformation for transgene expression. The SPFMV CP gene and SPLCV replication origin sequence are designed in antisense for the optimal induction of RNA silencing in transgenic sweetpotato. Research results showed that expression of foreign genes has been achieved by using *Agrobacterium tumefaciens* strain EHA105 harboring the expression cassette. Investigations on transgenic plant regeneration and resistance to virus infection under control conditions will be discussed.

### Identifying onion cultivars with reduced susceptibility to center rot

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Onion growers in Pennsylvania (PA) are continually challenged by losses from center rot disease caused by the bacterial pathogens *Pantoea ananatis* and *P. agglomerans*. The objective of this research is to identify onion cultivars that are less susceptible to center rot than the commercial standard cv. Candy but still meet the criteria of the PA Simply Sweet Onion Marketing Program. Thirteen onion cultivars were evaluated between 2015 and 2016 for center rot susceptibility and select horticultural characteristics, including neck and bulb diameter. Cultivars were arranged in a randomized complete block design and plots were split by proximity to inoculated plants. Two onion leaves of select plants were inoculated with a mixture of *P. ananatis* and *P. agglomerans* isolates. At harvest, onions were graded by size, marketability and disease incidence. Sub-samples of asymptomatic onions were evaluated for soluble solids, pungency and postharvest disease incidence. In 2015, six cultivars had marketable yields comparable to cv. Candy. Center rot incidence significantly differed between cultivars at harvest ( $P=0.002$ ) but not after 3-months in storage ( $P=0.241$ ). A positive, linear relationship was observed between late season onion neck diameter and center rot incidence. All cultivars met the minimum soluble solids and pungency criteria for the marketing program. Evaluations in 2016 will further investigate cultivars that show promise in sweet onion production.

### Early detection of adult plant resistance to oat crown rust using a qPCR assay

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Oat crown rust caused by the fungus *Puccinia coronata* f. sp. *avenae* is a major production constraint of oat in North America. There are over 100 major genes identified from various oat accessions, but their effectiveness is generally short lived. Adult plant resistance is the alternative and best option for durable crown rust resistance in oats. In field grown small grain cereals, adult plant rust resistance is usually measured at the flag leaf stage using a visual assessment of disease severity and infection type. Quantitative PCR-based assays have the potential to provide a less subjective measure of host resistance at an earlier stage in plant development. Such quantitative assay techniques provide a measure of the amount of fungus within infected plants which, for some rusts, can be directly extrapolated to host resistance. Here we report the use of a qPCR assay to detect adult plant resistance to crown rust in oats before the flag leaf stage in an experiment conducted in a growth chamber environment.

### Response of sweet sorghum lines to stalk pathogens *Fusarium thapsinum* and *Macrophomina phaseolina*

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Sweet sorghum [*Sorghum bicolor* (L.) Moench] has potential for bioenergy. It is adapted to a variety of U.S. locations and the extracted juice can be directly fermented into ethanol. However, little research on fungal stalk rots has been reported, even though these diseases pose serious constraints for yield, juice quality and biomass usability. A greenhouse bioassay was designed to assess charcoal rot (*Macrophomina phaseolina*) and *Fusarium* stalk rot (*Fusarium thapsinum*) susceptibility by maturity, when these diseases are manifested in the field. Multiple plantings of a susceptible grain line, RTx430, were used as a control for variation in flowering times amongst sweet sorghum lines. Lesion length measurements in inoculated peduncles were used to quantify disease severity. Sweet sorghum lines ‘Rio’ and M81E exhibited resistance to *F. thapsinum* and *M. phaseolina*, respectively, and in contrast, line ‘Colman’ exhibited susceptibility to both pathogens. Lesion development over time was monitored in Colman. These results will enhance molecular and biochemical analyses of sweet sorghum responses to pathogens, and breeding stalk rot resistant sweet sorghum.

### Development and evaluation of germplasm lines resistant to *Sclerotinia* stem rot

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*Sclerotinia sclerotiorum*, the causal agent for *Sclerotinia* stem rot (SSR), is a devastating fungal pathogen in soybean and reduced yield by 59 million bushels in 2009. Incomplete resistance in commercial cultivars can be improved through additional breeding efforts and understanding the genetic basis of resistance. SSR-resistant soybean germplasm was developed by crossing two sources of resistance, W04-1002 and AxN-1-55, with lines exhibiting resistance to other pathogens such as *Heterodera glycines*. After multiple screenings, 31 lines were selected for advanced SSR in field screens in 2014 and were down-selected to 16 lines with a high-level of multi-disease resistance in 2015. Germplasm lines 91-38, 51-23, and SSR51-70 exhibited SSR resistance with minimal lodging. Line 91-38 yielded 2605.5 kg/ha, 51-23 yielded 3156.2 kg/ha, and SSR51-70 yielded 2801.8 kg/ha. The highest yield achieved was 3748.8 kg/ha for 52-82B. While many of these experimental lines have excellent physiological resistance, yield and agronomic performance of these lines are not at the level of an elite commercial cultivar. For example, 91-38 yielded over 1000 kg/ha less than the best line in the trial, however it exhibited one of the lowest disease severity rankings. Germplasm lines such as 91-38, 51-23, SSR51-70, or 52-82B could be improved through further breeding to generate high-yielding commercial soybean cultivars with a high level of physiological resistance to SSR.

### Exploring the slow rusting potential of wheat genotypes against leaf and stripe rust of wheat

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The focus of current wheat research is to achieve durable rust resistance in Pakistan. We explored slow leaf and stripe rusting potential in 37 wheat genotypes by studying different slow rusting parameters, e.g., average coefficient of infection (ACI), latent period, pustule size, pustule density, infection



rate (IR), area under disease progress curve (AUDPC) and yield assessment (1000 grain weight and yield/ha). Multivariate statistical analysis techniques (Biplots and Heatmaps) were used to analyze and visualize the slow rusting parameters for identification of slow rusting genotypes. Genotypes including Lasani-2008, Chris, Crow, AS-2002, Punjab-2011, Frontana and FSD-85 demonstrated lower ACI, pustule density, IR, AUDPC, small pustule size, longer latent period, and higher 1000 grain weight and yield/ha against leaf rust. Genotypes encompassing Chris, AS-2002, Punjab-85, Yecora and Lasani-2008 revealed lower ACI, pustule density, IR, AUDPC, small pustule size, longer latent period, and higher 1000 grain weight and yield/ha against stripe rust. Combined analysis recognized genotypes including Uqab-2000, Yecora, Lasani-2008, Chris, Crow, Frontana and AS-2002 as slow leaf and stripe rusters. Hence, these slow rusters can be cultivated in fields for sustainable wheat production, and can be included in further breeding programs to produce durable rust resistant varieties.

#### **Resistance to *Claviceps purpurea* (Fr.) Tul. in the winter wheat lines “Robigus” and “Solstice”**

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Ergot, caused by *Claviceps purpurea*, is an important disease of cereals which can result in extensive financial losses to producers because of the presence of sclerotia containing toxic alkaloids in the grain. The severity of ergot in Canada and parts of Europe has increased over the past 20 years, and the development of wheat lines with resistance is being explored. The objective of this work was to determine if the winter wheat varieties “Robigus” and “Solstice” possess physiologic resistance to *C. purpurea*. Two winter wheat lines (“Robigus” and “Solstice”), two spring wheat lines (“Paragon” and “Cadillac”) and one durum wheat line (“Avonlea”) were inoculated with 5 Canadian and 4 UK isolates of *C. purpurea*. Nine spikes per wheat line were needle inoculated prior to anthesis for each pathogen isolate using a conidial suspension. Each spike was assessed for honeydew production 14 days after inoculation using a scale of 1 to 4; 1 = no honeydew to 4 = large drops running down the spike. The weight and number of sclerotia per spike were determined at plant maturity. “Robigus” had significantly lower honeydew production than “Solstice”, which had lower production than the other three lines. “Robigus” and “Solstice” also had significantly lower sclerotial weight and number per spike than the other three lines. “Robigus” and “Solstice” showed resistance to infection by *C. purpurea* compared to the other three wheat lines.

#### **Monitoring of Leaf rust in seventeen Egyptian wheat cultivars during 2013/2014 and 2014/2015 seasons**

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Wheat cultivars’ response to leaf rust caused by *Puccinia triticina* Eriks. vary between seasons; some cultivars lose resistance over time due to factors such as appearance of new pathogen races that have new virulence genes which overcome Lr-genes. This study monitored leaf rust development for adult plant stage of 17 Egyptian wheat cultivars, expressed in terms of infection type, final rust severity, area under disease progress curve and rate of disease increase (r-value) during 2013/2014 and 2014/2015 growing season in Sids research stations, Egypt. According to the results, only two varieties (Sakha94 and Misr1) exhibited adult plant resistance, three varieties (Gemiza9, and 10, and Misr2) were moderately susceptible, and twelve varieties (Sakha93, Giza168, and 171, Gemiza7, and 11, Sids1, 12, and 13, Shandwile1, and Bani-Swif1, 5, and 6) were susceptible during 2013-2014 and 2014-2015 growing seasons. However, nine varieties (Sakha94, Misr1, and 2, Giza 168, and 171, Gemiza 9, and 10, Sids 12 and 13) recorded low AUDPC up to 310 and the rest varied between 320:1120. Only five varieties (Sakha94, Misr1, Giza 168, and 171, and Gemiza 9) recorded r-value up to 0.092, while the rest ranged from 0.11 to 0.195 in both seasons. These data may help researchers and breeders by shedding light on the response of some Egyptian varieties to leaf rust in the last two growing seasons, and helping track the virulence genes in *P. triticina* during the same seasons.

#### **Scab susceptibility of a provenance collection of pecan**

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Scab (caused by *Fusicladium effusum*) is the most economically destructive disease of pecan in the Southeast US. Epidemics are favored by rainfall and high humidity. A provenance collection of ~950 pecan trees from 19 locations representing the native range of the species is located in Byron, Georgia, and was assessed for pecan scab severity in 1998, 2013 and 2014. Foliar scab severity was estimated visually. There were consistent significant differences among the 19 provenances ( $F = 5.6$  to  $62.5$ ,  $P < 0.0001$ ), with those from wetter locations (generally north of Texas) with the greatest proportion of scab resistant trees, while those provenances from the drier southern areas (Texas and Mexico) tended to be the most susceptible. However, within each provenance there was a range in susceptibility of trees. An association of provenance susceptibility with precipitation at the provenance source location bore out the relationship ( $r = -0.625$  to  $-0.823$  ( $P < 0.0001$  to  $0.004$ )). Estimates of heritability were not entirely consistent among years, but different methods were used to assess scab severity in 1998 (a 1-5 category scale) compared to 2013 and 2014 (the percent ratio scale). Despite using different methods, there was generally good agreement among years in regard to severity of disease on individual trees. In conclusion, trees from more northern populations (in areas with greater annual rainfall) are most likely to provide valuable and diverse sources of resistance to scab.

#### **Effectiveness of a seed plate assay evaluating charcoal rot resistance in soybean**

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Charcoal rot (*Macrophomina phaseolina*) of soybean (*Glycine max* (L.) is a disease of economic significance in the United States. While there is moderate host resistance in soybean cultivars, identifying and quantifying that resistance are difficult. Current assay methods, such as cut-stem inoculation and colony-forming unit index (CFUI), can be time consuming, and data may vary between tests. The objective of this research was to compare results of a seed plate assay to published results of the cut-stem and CFUI assays. Ten surface disinfested seeds of eight differential soybean genotypes, including LS94-3207, LS98-0358 and Pharaoh, susceptible and DT97-4290, DT98-7553, DT99-16864, DT99-17554 and DT99-17483 as resistant, were placed on water agar plates that had been inoculated with an isolate of *M. phaseolina* five days before. Plates were incubated at room temperature in the dark for 7 days and the number of seeds that germinated were determined. Noninoculated plates served as controls. In general, germination results from the plate assay were in agreement with the cut-stem and CFUI assays. Germination of resistance lines range from 58% to 84% and susceptible lines from 46% to 54%. Additional data will be presented later.

#### **Screening soybean germplasm for resistance to multiple *Fusarium* species**

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Fusarium root rot caused by multiple *Fusarium* spp. continue to cause increased soybean [*Glycine max* L.] yield losses in the United States. Commercial soybean varieties with complete resistance to *Fusarium* spp. are not available, thus necessitating the need for continued search for sources of resistance. The objective of this study was to identify screen soybean germplasm for resistance to four *Fusarium* species (*F. proliferatum*, *F. graminearum*, *F. sporotrichioides*, and *F. subglutinans*), which were identified as aggressive *Fusarium* spp. based on a preliminary study on soybean in South Dakota. To evaluate soybean germplasm for resistance to the four *Fusarium* spp., 44 genotypes belonging to maturity groups I from 10 different countries were

screened in replicated tests using the inoculum-layer method. Disease severity was evaluated at 14 days after inoculation as a percentage of lesion produced on soybean roots by the causal pathogen. Preliminary results suggests one genotype (PI 603371) had significantly lower disease severity ( $P < 0.05$ ) than the susceptible check for all the four *Fusarium* species. This genotype will be useful as a parental material to develop commercial soybean cultivars with resistance to *Fusarium* species.

#### **Development of an *in vitro* bioassay to screen perennial Phlox germplasm for susceptibility to *Golovinomyces cichoracearum* (*Erysiphe cichoracearum*)**

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Phlox is a genus of perennial and annual plants with colorful, long-lasting flowers, and a common inhabitant of gardens worldwide. In humid and warm areas, its cultivation is severely limited by powdery mildew. Published studies on resistance of Phlox to powdery mildew have mainly focused on the species *subulata*, *paniculata* and *drummondii*. However, the genus includes many more species, some of which could be valuable from both an ornamental and resistance standpoint. This project aims to develop a reliable laboratory bioassay to conduct *in vitro* screenings of Phlox germplasm for susceptibility to powdery mildew. The project is in collaboration with the OSU Ornamental Plant Germplasm Center, which allows access to more than 65 species of Phlox. For this purpose, detached leaves of healthy Phlox were disinfected and plated on water-agar medium. Variations of the host species, media type, disinfection process, and inoculation technique were evaluated. Disinfected leaf tissues remained in good status and contaminant-free for up to one month. Infection and growth of the pathogen on the host required specific conditions of light, temperature and relative humidity. To the best of our knowledge, this study represents the first attempt to develop a laboratory bioassay to screen Phlox resistance to powdery mildew. This assay has the potential to represent a useful tool for screening resistant lines and for phytopathological studies on the biology of the pathogen.

#### **Virulence of *Calonectria pseudonaviculata* and *C. henricotiae* isolates and screening for resistant boxwood cultivars in detached leaf assays**

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Boxwood blight caused by the two *Calonectria* species, *C. pseudonaviculata* and *C. henricotiae*, is a recently emerged threat to the annual \$1.3 million boxwood industry. While fungicidal control can be effective, breeding resistant or tolerant cultivars is the preferred long-term strategy to control the disease in the landscape. Developing an effective assay to quickly screen potentially thousands of boxwood genotypes is an essential first step in such a breeding program. Therefore, this study was conducted to evaluate the virulence of different *Calonectria* isolates on a susceptible boxwood host. The virulence of 24 isolates was determined by inoculating detached leaves of *Buxus sempervirens* ‘Suffruticosa’ using two different inoculation techniques. All 24 isolates were virulent on ‘Suffruticosa’, but the virulence varied among isolates. The isolate NCBB-1 was the most virulent, and was used to compare relative resistance among eight diverse boxwood taxa. Preliminary results indicate that two boxwood taxa, *B. harlandii* and *B. sinica* var. *insularis* ‘Nana’, are the most tolerant to this isolate in detached leaf assays.

#### **Evaluating nine boxwood cultivars for susceptibility to *Calonectria pseudonaviculata* and *C. henricotiae***

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Boxwood blight is caused by two species of *Calonectria*: *C. pseudonaviculata* (Cps) and *C. henricotiae* (Che). In North America, only Cps is known to occur, but the 2nd species is of concern since there is evidence that it differs in its temperature optimum, pesticide sensitivity and tolerance to sanitizers. US boxwood cultivars are currently being evaluated for tolerance to Cps and it would be useful to know if their reaction to Che is similar. Based on a previous assessment of susceptibility of 42 boxwood cultivars to Cps, nine were selected on a spectrum from highly susceptible to relatively tolerant. Cultivar sets were inoculated with spore suspensions (mixtures of 4 representative isolates) of each species and the experiment was repeated three times. While results showed some differences in cultivar behavior from trial to trial, there were cultivars that were consistently susceptible (‘Dee Runk’, ‘Vardar Valley’) or consistently tolerant (‘SB17’, ‘Little Missy’) and the interaction ‘pathogen type\*’cultivar’ was not significant. This suggests that breeding for tolerance for one species will confer tolerance for the other.

#### **Susceptibility of *Buxus* accessions to the boxwood blight pathogen *Calonectria pseudonaviculata***

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Forty *Buxus* accessions from the National Boxwood Collection of the U.S. National Arboretum provided as potted plants were evaluated for susceptibility to boxwood blight caused by *Calonectria pseudonaviculata* (Cps). Two experiments were conducted with 3 to 5 replicate plants each. Plants were inoculated with  $1.5 \times 10^5$  Cps conidia in 2 ml coarse spray per plant and grown under high humidity with overhead irrigation. Leaf and stem lesions were recorded 3 or 4 wks after inoculation. *B. harlandii* (1 accession), *B. sinica* (3), *B. microphylla* (7) and *B. bodinieri* (1) had fewer lesions per plant than *B. microphylla*  $\times$  *sempervirens* (6), which had fewer lesions than *B. sempervirens* (20 accessions). *B. wallichiana* (1 accession) was the most susceptible species (>160 lesions). Variation within species was also observed. *B. sinica* var. *aemulans* (accession 60705\*H), *B. sempervirens* (36365\*J) and *B. harlandii* (18834\*H) were the least susceptible with < 10 lesions per plant. *B. sempervirens* ‘Scupi’ (9548\*H), *B. microphylla* ‘Compacta’ (4899\*CH), *B. sempervirens* ‘Arborescens’ (57953\*H), *B. sinica* var. *insularis* ‘Pincushion’ (51898\*H) and *B. microphylla* var. *japonica* ‘Jim Stauffer’ (72213\*H) each had < 20 lesions. These data differ from previous detached leaf and unrooted cutting assays. Plant size may affect inoculation and disease, normalizing did not result in significant differences in susceptibility ranking. These results may aid in the development of boxwood resistance to Cps.

#### **Identification of *Brassica napus* plant introductions with resistance to pathogenicity group 4 of *Leptosphaeria maculans***

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Blackleg caused by *Leptosphaeria maculans* (Desmaz) Ces. & de Not is a serious disease of canola (*Brassica napus* L.) worldwide. In North Dakota, *L. maculans* isolates that overcome resistance genes *Rlm1*, *Rlm2*, and *Rlm3* are the most prevalent and have been classified in pathogenicity group (PG) 4 using a set of three differentials. The objective of this study was to identify sources of resistance against PG-4. Replicated greenhouse trials using mixtures of five PG-4 isolates were used to evaluate the reaction of *B. napus* plant introductions (PI) at the seedling stage. Twenty one PI materials identified as resistant were evaluated in field trials in 2014 and 2015 using a randomized complete block design with four replications. Cultivar ‘Westar’ and two commercial canola hybrids were used as controls. Seedlings were inoculated repeatedly with a mixture of spores from the same five isolates used in the greenhouse and supplemented with naturally infested residues. Disease severity was estimated at harvest as percentage of internal stem crown tissue discoloration. Five PIs were considered highly resistant and behaved consistently better ( $P < 0.05$ ) than the hybrids in both field trials with an average disease severity < 4% compared to 25% of the hybrids. Crosses between these PIs and canola breeding lines have been made to transfer the resistance into advanced germplasm. In addition, efforts to produce doubled haploid mapping populations from these sources are under way.

### **Evaluating chemical fingerprinting as a tool to rapidly screen hybrid chestnut for resistance to pathogens**

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Chestnut blight, caused by the introduced pathogen *Cryphonectria parasitica*, continues to plague American chestnut (*Castanea dentata*) in the eastern US. Restoration is focused on breeding hybrids that morphologically resemble American chestnut but contain blight resistance from Chinese chestnut (*C. mollissima*). Compounding the problem for restoration efforts, *Phytophthora cinnamomi*, an agent of root rot, has emerged as a major pathogen in chestnut's southern range. Time and resource intensive inoculation-based screening methods are used currently to identify chestnuts resistant to these pathogens. Therefore, we evaluated if chemical fingerprinting (CF), a potentially less resource-intensive tool for phenotyping resistant/susceptible trees, can be used to screen chestnut for disease resistance. A Varian 3100 Fourier-transform infrared (FT-IR) spectrometer equipped with an attenuated total reflectance accessory was used to fingerprint methanol extracts from 0-1 year old chestnut stem tissue that differed in susceptibility to chestnut blight. Using Soft Independent Modeling of Class Analogy, chemical differences among disease rating classes were identified and used to develop class discriminating models. Early results suggest that CF may be a useful tool for screening seedlings for blight resistance; however, we are currently testing additional samples to refine, optimize, and validate models for both diseases.

### **Screening soybean germplasm for resistance towards *Pythium* species**

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*Pythium* species are a major cause of soybean seedling root rot and seed rot in Ohio. One method of management is the use of resistant cultivars. Previous studies identified a "differential" resistance response to *Pythium ultimum* var. *ultimum* and *P. ultimum* var. *sporangiferum* among soybean cultivars. The purpose of this experiment was to quantify the resistance level of several germplasm lines and to identify any potential "differential" effect. To do this, several soybean germplasm lines were evaluated for resistance to *Pythium* isolates collected from fields in Ohio using a seed plate assay. Seed plate assay results indicated that Conrad, Dennison, Sloan, and Kottman all had varying levels of resistance towards 13 species of *Pythium*; more importantly, there was a highly significant ( $P < 0.0001$ ) cultivar x *Pythium* spp. interaction. All cultivars were very susceptible to *P. ultimum* var. *ultimum*, *P. ultimum* var. *sporangiferum*, and *P. sylvaticum*. When inoculated with *P. conidiophorum*, *P. atrantheridium*, and *P. dissotocum*, Conrad, Dennison, and Lorain had a lower degree of seed rot than either Sloan or Kottman. These resistant cultivars may be used to provide a higher degree of protection against infection from *Pythium* species in soybean seeds and seedlings.

### **Disease susceptibility screening for cold-climate wine grape cultivars**

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The wine grape industry in the Upper Midwest has expanded rapidly in the past 15 years. Inconsistent reports on disease susceptibility in cold-climate wine grape cultivars may be leading to overspraying and underutilization of host resistance in the region. Inconsistencies in reports may stem from a lack of study on adult plants in a randomized, replicated, and fungicide free vineyard environment. Two vineyards containing eight cultivars were left unsprayed by fungicides in 2015. Severity of downy mildew, powdery mildew, and black rot was measured from bud break until 2 weeks post-harvest. There was a significant difference among cultivars for all three diseases at both sites. A second assay was conducted using potted grape vines as "trap plants" to capture inoculum present in the field and gain further information on disease susceptibility. Using trap plants and artificial humidification did not generate results consistent with the disease severity and progress observed in the field during the growing season. These preliminary data suggest that selection of certain cultivars has the potential to reduce sprays needed to manage diseases during the growing season in the Upper Midwest. Results further indicate differences exist between foliar and fruit susceptibility for all three diseases, a distinction that is not made in current rating systems. This work will continue in 2016 to gain additional susceptibility data and observe differences between growing seasons.

### **Evaluation of stem inoculation technique for assessing resistance to *Phytophthora nicotianae* in hydrangea cultivars**

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Almost all hydrangeas, especially container-grown oakleaf hydrangeas (*Hydrangea quercifolia*) are more susceptible to *Phytophthora* root rot disease. Most economic and trading hydrangea cultivars are propagated as rooted cuttings. Reliable and rapid stem cutting-based screening technique to evaluate hydrangea cultivars for resistance to *Phytophthora nicotianae* is needed toward disease management. Hydrangea cvs. Queen of Hearts, Ruby Slippers and Munchkin stem cuttings were screened in a laboratory procedure by point inoculation with 5-day-old culture, #1 size (outside diameter 5 mm) plugs of *P. nicotianae*. Each stem cutting was evaluated for lesion area and lesion severity after 3, 5 and 7 days of incubation at 25°C with 16-hr-light/8-hr-dark cycle. The experiment was repeated twice. After comparing the lesion development on three hydrangea cultivars, the results showed that Queen of Hearts is most susceptible to *P. nicotianae* than other cultivars and five days observation is the optimal time for stem cutting-screening technique. This study will facilitate the ongoing large-scale screening for *Phytophthora* root rot resistance among hydrangea cultivars very easily and it is clear that described stem cutting-inoculation technique has the potential to play a significant role for simple, efficient and reproducible screening.

### **Cavity spot of carrots: Progress in screening for resistance**

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Cavity spot of carrot is caused by several *Pythium* species, especially *Pythium violae* and *P. sulcatum*. Field trials were conducted from 2013 to 2015 to screen 60 carrot lines from the USDA breeding program at the University of Wisconsin lines for differences in susceptibility to cavity spot. Cultivars Atomic Red, Cellobunch and Envy were included as commercial checks. Carrots were seeded into 'muck' soil (pH 5.7- 6.5, organic matter 65- 78%) where the cavity spot pathogens occur naturally. Carrots were direct seeded ( $\approx 75$  seeds/m) in early June, onto raised beds, 66 cm apart and 6 m in length. Fifty carrots per replicate were harvested in late October, placed in cold storage for 6 – 8 weeks and washed and assessed for cavity spot incidence and disease severity (DSI). Cavity spot incidence and severity were very high in 2013 (97% and 69 DSI) and 2014 (96%, 54 DSI) and lower in 2015 (50%, 26 DSI). A wide range of susceptibility to cavity spot was found each year. Purple lines 6139B, 6244B, 7262B and 3497B were highly resistant (1- 8%, 1-7 DSI). Two orange carrot lines, in the trials in 2014 and 2015, had consistently low disease. Line 1137B had 8% cavity spot in both years, with DSI of 7 in 2014 and 4 in 2015. Line 5367 B had 14 and 21% cavity spot in 2014 and 2015, respectively, with a DSI of 8 and 7. Field screening has identified breeding lines with promising levels of resistance to cavity spot.

### **Screening of Wild Hop (*Humulus lupulus*) Germplasm for Identification of Resistance to *Pseudoperonospora humuli***

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*Pseudoperonospora humuli*, the causal organism of hop downy mildew, is considered the most economically-damaging pathogen to hop production. Host resistance, in addition to intensive cultural management practices and fungicide use, is the primary control measure. Wild hops (*Humulus* spp.) are

distributed across the North America and Eurasia. They may provide a resource for novel resistance genes and germplasm diversity for local hop breeding programs. Sixteen sites in Minnesota with wild populations of hops were identified. At 14 of these sites individual rhizomes were recovered from 1 – 27 unique individuals. One-hundred six wild and cultivated hop genotypes originating from the USA, England, Japan, and Kazakhstan were screened for foliar resistance to downy mildew (*Pseudoperonospora humuli*) using a detached-leaf assay. Each genotype was tested with three replicates. In total, 33 genotypes were as resistant as the current commercial standard (cv. ‘Teamaker’) which included five other cultivars, five Japanese hop (*H. japonicus*) genotypes, two experimental breeding lines, and 21 wild *H. lupulus* genotypes. We identified two geographically distinct locations in Minnesota containing previously unreported germplasm possessing high levels of foliar resistance that may be useful for breeding locally-adapted varieties. Work is currently underway to characterize the morphological characters and chemical attributes of the hop inflorescences of selected resistant wild plants.

#### **Barley yellow dwarf incidence and bird cherry-oat aphid preference in four wheat varieties in Idaho**

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Barley/Cereal yellow dwarf viruses (B/CYDV) are aphid-borne pathogens that are transmitted by several species of cereal aphids, chief among them the bird cherry-oat aphid (BCOA), *Rhopalosiphum padi* (L.). This species has also been linked to the recent consecutive outbreaks of BYDV-PAV, between 2013 and 2015, in southern Idaho. The present study was conducted to evaluate the relative susceptibility of four commonly planted winter wheat varieties in Idaho, namely, SY Ovation, Brundage, Stephens, and WB-Junction, to BYDV and its BCOA vector. In choice experiments, viruliferous aphid preference for the four varieties was assessed by counting the number of aphids per plant 24 hours after infestation. BYDV titer within seedlings was quantified using qPCR, three weeks after inoculation. During a two-year field-cage study, BYDV symptom severity, aphid pressure, and root and shoot biomass were assessed for the four varieties. Root/shoot biomass data are currently being analyzed. BYDV in experimental cages was confirmed using ELISA. Although Stephens and SY Ovation were preferred relatively less frequently than WB-Junction and Brundage by BCOA, no significant differences were detected in aphid preference among the four varieties. Likewise, BYDV incidence in field cages was not statistically different among the four varieties, however SY Ovation had the lowest BYDV incidence, an observation consistent across both years of the study.

#### **Resistance in pea to the *Bean leaf roll virus***

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*Bean leaf roll virus* (BLRV) is a major luteovirus impacting dry pea production on an annual basis in Idaho and Washington. Symptomatic plants are often chlorotic, stunted and distorted. The virus is transmitted by aphids in a persistent manner and cannot be mechanically transmitted. Host resistance is an important economic and environmentally favorable means to manage this pathogen. Pea aphids were used to phenotype the genetic resistance of 36 pea genotypes, mostly consisting of dry pea cultivars, under controlled greenhouse conditions. Two pea genotypes, Journey and Stirling, were determined to have complete resistance to BLRV. Journey is a commercial forage green dry pea and Stirling is a formerly grown commercial green dry pea. Three commercial pea cultivars, Majoret, Granger and Spector were determined to be highly susceptible to BLRV with a 100% incidence of infection across three tests. Mapping populations will be established between the resistant and susceptible pea genotypes to identify molecular markers that can be used in marker-assisted selection of pea germplasm for genetic resistance to BLRV.

#### **The SimpleSynteny Server: Genome Comparison Simplified**

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Visualizing syntenic relationships amongst gene clusters is a common task for studying organismal evolution. Here we present SimpleSynteny, a new comparative genomics web server which includes a number of helpful tools for the analysis of microsynteny across multiple genome assemblies. Requiring only FASTA files for genomes and gene targets of interest as input, SimpleSynteny’s easy interface helps the user explore and customize individual genomes before combining them into a final high-resolution figure. The approach gives users the freedom to incorporate additional knowledge when editing contigs/scaffolds after computational predictions are made. A range of image formats and customization options are also provided to give additional control when saving results. In this presentation, we provide a quick overview of the tools provided and generate example figures from fungal genomes via a live demonstration of the site. SimpleSynteny stands to be a powerful new tool in a researcher’s toolbox of comparative genomics software. The server is free to use and is currently available at: <http://www.simplesynteny.com>.

#### **Reducing Mycotoxin Levels in Corn via Lepidopteran Insect Control and Application of Atoxicogenic Fungi**

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Mycotoxins are secondary fungal metabolites that have adverse health effects on animals and humans. Lepidopteran damage to corn is suspected to facilitate fungal colonization and infection to kernels and to increase the risk of mycotoxin production and accumulation in corn. This study was initiated to evaluate the application of AF-36 and Afla-Guard at two growth stages in reducing mycotoxin levels in corn. Treatments included an untreated control, insect control, AF-36 and Afla-Guard applied at the V8 or VT growth stage, and Afla-Guard applied at the V8 or VT growth stage with insect control. The application of AF-36 or Afla-Guard applied at the V8 or VT growth stage did not significantly reduce the concentration of either aflatoxin or fumonisin. In 2013, the insect control significantly reduced the fumonisin concentration, and in 2014 application of insecticide alone or with Afla-Guard applied at the V8 or VT growth stage reduced the concentration of fumonisin. Aflatoxin levels were not significantly reduced by the application of AF-36 or Afla-Guard at either application time or by the insecticide applications. Fumonisin concentrations in both years showed a positive relationship with the amount of ear damage caused by *Helicoverpa zea* and *Spodoptera frugiperda*. Aflatoxin concentrations in 2013 were positively correlated to the amount of ear damage, but no such correlation was observed between aflatoxin and insect damage in 2014.

#### **Deciphering soil and plant microbiomes associated with suppression of soybean diseases**

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General disease suppression of soilborne pathogens can add to the toolbox of strategies for disease management. This study was undertaken to identify the microbiome indicators for soil suppressiveness to sudden death syndrome (SDS) of soybeans. Five cover crop treatments (rye, vetch, mustard, rye+vetch, fallow) and two tillage methods (chisel plow, ridge till) were compared. Each cover crop plot was divided to receive both tillage treatments, and there were four replications of this experiment. Soybean plants were evaluated for naturally occurring foliar and root diseases at the R6-R7 growth stage. Root, rhizospheric soil, and bulk soil samples were taken early in the season, and DNA was extracted for PCR amplification, targeting 16S bacteria and archaeal, and ITS genes, for subsequent high throughput sequencing. Our results have shown no significant differences in microbial populations of the cover crop treatments. However, there are several notable differences with the tillage treatments. Chisel plowed plots had increased levels of the

genera, *Verrucomicrobia*, *Glomeromycota*, *Proteobacteria*, and *Actinobacteria*. Ridge tilled plots showed increased levels of *Streptophyta*, *Firmicutes*, and *Crenarchaeota*. These results are being verified with a greenhouse assay to determine if it is possible to delineate microbiome profiles that correspond to general disease suppression.

#### **Effects of fungicide application timing and cultivar resistance on Fusarium head blight and deoxynivalenol in winter wheat**

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*Fusarium graminearum* causes Fusarium head blight (FHB) in wheat. FHB reduces yield and quality and contaminates grain with the mycotoxin deoxynivalenol (DON). Effective management strategies are needed. The objectives of this research were to 1) Determine the effect of fungicide application timing at anthesis (the standard timing) and 6 and 12 days later on FHB and DON in the winter wheat cultivars Overley (susceptible) and Overland (moderately resistant) and 2) Compare the effects of a triazole and a strobilurin fungicide on FHB and DON in Overley and Overland. In 2015 two field trials (irrigated and rain-fed) were conducted in Nebraska, USA. The triazole Prosaro (prothioconazole + tebuconazole) and the strobilurin Headline (pyraclostrobin) were applied with a CO<sub>2</sub>-powered backpack sprayer at anthesis and 6 and 12 days later. A split plot design in randomized complete blocks with 4 replications was used. Main plots were cultivars and subplots were fungicide treatments. FHB index and DON were significantly ( $P < 0.05$ ) lower in Overland than in Overley. The window of fungicide application to control FHB and DON was widened from anthesis to 6 days later without loss of efficacy. Headline was less effective than Prosaro in controlling FHB and DON. Moderate resistance combined with a triazole fungicide most effectively reduced FHB and DON. The results indicate a wider fungicide application window and the effectiveness of combining resistance with a triazole fungicide.

#### **Impact of Agronomic Practices and Diseases on Wheat Profitability**

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Changes in nitrogen fertilizer rates, row spacing, and fungicide programs have been proposed as strategies for increasing wheat productivity and profitability in Ohio. The main goal of this study was to evaluate the economic benefit of these practices as influenced by diseases. Field experiments were conducted in 2013, 2014 and 2015 using a split-split-plot design with nitrogen rates (N-rate) as whole-plot, row spacing (19 and 38 cm) as sub-plot, and Prosaro fungicide treatments (TRT) as sub-sub-plot. Combinations of these factors represented separate management programs (PROG), and an economic analysis was conducted using yield (YLD), test weight (TW), grain prices and price discounts due to poor grain quality, as well as fertilizer, seed and fungicide costs to estimate net cash income for each PROG. Linear mixed model analyses showed that leaf rust and Fusarium head blight (FHB) were higher and YLD and TW lower in wide than in narrow rows. Leaf rust, YLD, and TW increased with increasing N-rates, but these effects varied with TRT. FHB severely affected the economic benefit of the different PROG. Compared to the standard PROG used in Ohio (100.9 kg N ha<sup>-1</sup>, narrow rows and untreated), several of the modified PROGs only resulted in greater economic benefit (\$3 to 215 ha<sup>-1</sup>) when FHB levels were low. PROGs with narrow rows plus high N rates (> 134 kg N ha<sup>-1</sup>) and a Prosaro treatment were among the most profitable, while wide-row PROGs were among the least profitable.

#### **Effect of cropping rotation and input level over 18 years on the northern Great Plains**

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A cropping rotation study was conducted from 1994-2012 (three 6-yr cycles) at the Agriculture and Agri-Food Canada Research Farm at Scott, Saskatchewan, Canada in a split-split-plot design with four replicates. The main plots were three levels of inputs; high (HIGH), reduced (RED), and organic (ORG) The subplots were three levels of cropping diversity (rotations); fallow-annual grains (LOW), diversified annual grains (DAG), and diversified annuals and perennials (DAP). The sub-sub plots (each 13 × 40 m) were phases of the crop rotation. Foliar disease assessments were made shortly before crop maturity each year. Across treatments and years, there was no consistent pattern of response of final disease severity to treatment. For example, level of input and cropping diversity did not affect severity on the penultimate or flag leaf of wheat in 2007-2013. In those years where there were differences in severity, severity was often highest in the high-input treatments, but organic rotations were highest in some years. However, foliar disease severity differed substantially among years. This study demonstrated that weather conditions on the northern Great Plains had a much larger impact on foliar disease severity than input levels or cropping rotation in the moderate to highly diverse rotations assessed in this 18-year study.

#### **Effect of root pathogens *Bipolaris sorokiniana* and *Fusarium graminearum* on germination and seedling blight in wheat in South Dakota**

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*Bipolaris sorokiniana* (Bs) and *Fusarium graminearum* (Fg) are important wheat root pathogens that effect seed germination and seedlings establishment thus ultimately impact the crop productivity. The objective of this study was to explore the effect of Bs and Fg infested seed on germination and seedlings establishment (blight) of 7 wheat cultivars, Advance, Briggus, Forefront, Oxen, Russ, Prevail, and SD4215 which were planted at Volga, SD in 2015. The treatments included, uninfested + untreated, uninfested + treated with fungicide, infested (Bs) + treated, infested (Bs) + untreated, infested (Fg) + treated, infested (Fg) + untreated. Seed germination and seedling blight data was recorded after the germination for 3 consecutive weeks. Wheat cultivars varied in seed germination and seedling blight to both pathogens; low seed germination (ranging from 35-71%) and high seedling blight (30-65%) was observed in Fg infested seed, whereas, in Bs infested seed the germination ranged 58-100% and seedling blight ranged 0-42%. The cultivars Oxen and Prevail had the lowest (33%) and the highest (71%) seed germination respectively. Also the treatments including the fungicide significantly increased the germination (ranging from 5-44%) and the seedling blight got reduced in almost all the cultivars.

#### **Evaluation of management programs of yellowing and wilting of blackberry (*Rubus* sp.) caused by *Fusarium oxysporum* in Michoacan Mexico**

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Yellowing and wilting of blackberry (*Rubus* sp.), caused by *F. oxysporum* is one of the most devastating diseases in Michoacan, Mexico. The objective of this research was to evaluate different disease management programs using biological control agents (BCA), plant activators, and fungicides. The experiment was established in 2014 and 2015 in a plantation of blackberry cv. Tupy. A randomized complete block design with 4 blocks and 13 treatments and a control were used. Treatments consisted of sequential applications of fungicides (prochloraz, difenoconazole, thiabendazole, azoxystrobin and methyl thiophanate), plant activators (acibenzolar-S-methyl, potassium phosphite, oligosaccharin-glutathione), BCA (*Trichoderma harzianum*, *Streptomyces lydicus* strain WYEC 108) and microbial mixtures (Maya Magic<sup>®</sup> and Microsoil<sup>®</sup>). Area Under Disease Progress (AUDPC) of incidence, severity and plant mortality were assessed. There were significant differences between treatments. Treatments with the lowest AUDPC were the mixtures of microorganisms (Maya Magic<sup>®</sup>) without significant differences with treatments beginning with 2 to 3 applications of prochloraz or difenoconazole followed by Glutathione-oligosaccharins, and azoxystrobin, followed by acibenzolar S methyl applications. These results indicate that it is feasible to significantly reduce the severity and mortality in *F. oxysporum*-infected blackberry plantings, if disease management programs begin early in the growing season.

### **Response of grafted seedless watermelon to root regeneration and inoculation with *Fusarium oxysporum* f. sp. *niveum***

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Grafting can be a successful tool in an integrated approach to manage soilborne diseases such as Fusarium wilt of watermelon caused by *Fusarium oxysporum* f. sp. *niveum* (FON). In cucurbit grafting, excision and regeneration of grafted seedling roots is often practiced by nurseries. The objectives of this greenhouse experiment were to assess the performance of grafted seedless watermelon plants under inoculation with FON, and compare grafted plants with original rootstock roots and with root regeneration. 'Melody' watermelon was grafted onto two squash rootstocks including 'Marvel' and 'Super Shintosa'. Various plant growth parameters were measured. All the plants were plated on PDA to confirm the presence of FON colonies 32 days after transplanting. Non-grafted watermelon plants showed a significantly ( $P \leq 0.05$ ) higher percentage of recovered FON colonies (75%) compared to grafted treatments (0-8%). Plant growth measurements indicated less vigorous growth for non-grafted plants when compared to the grafted treatments. Root regeneration treatments had a numerically higher percentage of FON colonies (0-8%) compared with the regular grafting treatments (0-3%), while no differences were observed in plant growth measurements. These results showed that grafting can effectively prevent FON colonization in seedless watermelon, and supported the use of this practice as part of a watermelon production integrated management program.

### **Novel approaches for the integrated control of the soilborne strawberry pathogens *Macrophomina phaseolina* and *Fusarium oxysporum* f. sp. *fragariae***

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*Macrophomina phaseolina* (Mp) and *Fusarium oxysporum* f. sp. *fragariae* (Fof) are emerging soilborne pathogens causing crown rot and Fusarium wilt in commercial strawberry fields in California. Fungicides representing eight active ingredients from four different FRAC groups were evaluated for their efficacy against each pathogen in greenhouse bioassays. Twenty-four fungicide treatments (eight fungicides at three rates) were drench applied to pathogen infested potting media two days prior to planting and 19 days after planting. Tea bags of buried inoculum were recovered two days after each fungicide application and plated on semi-selective medium to quantify each pathogen; disease severity was assessed weekly. Differences in the colony forming units (CFUs) and disease severity (AUDPC) for all treatments and the inoculated water-drench control for both Mp and Fof were not statistically significant, although there were differences among the CFUs for Mp. In addition, a strawberry plant extract was assessed for its ability to stimulate germination of Mp microsclerotia *in vitro*. Preliminary results showed that the number of germinating microsclerotia was significantly higher after the application of the strawberry extract. Germinated microsclerotia may be more sensitive to fungicides and offer a means of increasing fungicide efficacy against soilborne pathogens.

### **Correlating the Effects of Glyphosate on Soybean Nutrition with Cercospora Leaf Blight and Septoria Brown Spot**

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Glyphosate is linked to detrimental effects on soil microbiota and reduced levels of metal cations available for plant uptake. There is no agreement in literature regarding effects of glyphosate on plant diseases or micronutrient uptake. Some authors claim the use of glyphosate in Roundup Ready (glyphosate-resistant) soybeans leads to increased disease severity due to negative effects on uptake of manganese (Mn), iron (Fe) and other micronutrients, while others dispute these findings. This controversy is relevant to findings made by our laboratory regarding tissue concentrations of certain micronutrients, e.g. Mn and Fe, which substantially affect *Cercospora* leaf blight (CLB) in soybean. Therefore, we tested the hypothesis that glyphosate is affecting disease severity in soybean. Twelve Roundup Ready soybean varieties were planted at two locations in Louisiana in randomized complete block designs. Plots were either not sprayed or sprayed with Roundup. CLB and Septoria brown spot disease assessments were conducted in 2014 and 2015. Samples were taken for micronutrient analyses and real-time quantitative PCR (qPCR) for the CLB pathogen. Results showed significant increases of some elements, e.g. N and Mn, for Roundup-treated samples. CLB and Septoria brown spot severities were higher in non-treated plots. Results from qPCR analyses indicated more pathogen DNA (biomass) in Roundup-treated samples, however, these findings were not correlated with disease severity.

### **Decision models for fungicide applications for frogeye leaf spot in soybean**

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Frogeye leaf spot (FLS), caused by *Cercospora sojae*, is a common foliar pathogen of soybean in the United States and other soybean production areas in the world. While foliar fungicides can reduce disease severity and yield loss caused by FLS on susceptible cultivars, the appropriate application timing is essential for optimal fungicide efficacy and yield protection. To better manage FLS epidemics in Tennessee, fungicide application decision models were developed and evaluated across locations in 3 different counties in 2013 and 2014. Application models were developed based on growth stage of soybean and rain/irrigation events or temperature and relative humidity parameters. The number and timing of fungicide applications varied by location, year, and application model. All applications reduced disease severity, but only applications under moderate to severe disease pressure were necessary to protect yield. Results indicate models for fungicide application timing for FLS management need to incorporate additional disease parameters to more accurately trigger fungicide applications based on environmental factors and soybean growth stage.

### **Controlling Asian Soybean Rust and Phytophthora Late Blight by Biologicals Alone and in Combination with Fungicides**

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Tremendous losses are associated with Asian soybean rust (*Phakopsora pachyrhizi*) and Phytophthora late blight (*Phytophthora infestans*) yearly. At AgBiome, isolates from our large collection of proprietary environmental microbes are screened in high-throughput assays against a broad range of fungal pathogens, insect pests, and plant-parasitic nematodes. Genome sequences from these isolates are analyzed for known families of proteins active against plant pests, and these proteins are assayed in bacterial expression systems and transgenic crops. Successful biological and trait candidates are tested, and confirmed active biologicals proceed to the fermentation and formulation. Nearly 1200 and 500 isolates have been screened against *P. pachyrhizi* and *P. infestans*, respectively. For both pathogens, the initial screening is done using leaf disks and hits are confirmed on whole plants in growth chambers. Selected leads undergo mouse toxicity testing and scale up by fermentation and formulation before they are evaluated in greenhouse and field. From all isolates screened for the control of Asian soybean rust, seven were selected and were evaluated in the greenhouse in Florida and Illinois and in the field in Florida and Argentina. Additional selected leads with activity against *P. infestans* will be sent to the field this year.

### **Proactive Strategy for Management of Seedborne Pathogens of Pulse Crops in Montana**

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Pulse crops (chickpea, lentil, and field pea) are grown for their health and nutritional benefits. In 2015, Montana led the U.S. in field pea and lentil production at 48% acreage. The pulse crop acreage projection by 2019 is 1.4 mil acres. Growers in Montana are aware of the fungal disease *Ascochyta/Mycosphaerella* blight and have been testing their seeds exclusively for this disease complex. Due to limited seed availability, seed is coming into the state from other long-term pulse producing areas with pathogens. To determine the prevalence of seedborne fungi in pulse crops from the 2015 growing season, seed samples of field pea (256), lentil (170), and chickpea (35) were tested by plating 400-600 seeds per sample on potato dextrose agar and incubating at 20°C (12h light: dark) for 10-14 d. Fungi were identified to genus level using microscopy and DNA sequencing. The most prevalent fungi isolated were *Alternaria* (71 - 83%), *Cladosporium* (53 - 67%), *Ascochyta* (18-56%), *Penicillium* (46 - 57%), *Rhizopus* (20-40%), and *Nigospora* (6-20%; 1<sup>st</sup> report). Others were *Fusarium* (14- 17%), *Aureobasidium* (7.4-11%; 1<sup>st</sup> report), *Botrytis* (8-14%), *Stemphylium* (9 - 16%), *Colletotrichum* (1-9%), and *Diaporthe* spp. (4-14%; 1<sup>st</sup> report). Growers can manage these fungal diseases if they know the health status of their seedlots, use seed treatment fungicides, and prevent planting seed lots infested with pathogens with long-lived survival structures on organic and conventionally managed acres.

#### **Production practices impact yield parameters and disease severity on sweet and forage sorghum grown as biomass feedstock**

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Sweet and forage sorghum are potential feedstocks for energy and chemical industries but the impact of production practices on diseases is largely unknown. Planting date (PD), nitrogen (N) rate, and variety impact were assessed in 2011 and 2012 on plant populations, above ground dry matter (ADM) yield, total fermentable sugar concentration (°Bx), and anthracnose severity. A factorial with year as the main plot, PD as the split-plot, N rate as the split split-plot, and sweet and forage sorghum variety as the split split split-plot was used. Disease assessment and harvest was at soft dough. Plant population was not impacted by any variable. ADM yield segregated by year, PD, and variety. Generally, similar ADM yield was noted at all N rates. M81-E sweet sorghum ADM yield trended higher than forage sorghum. °Bx varied by year, PD, and variety with values declining at the later PD. M81-E had a higher °Bx than either forage sorghum with 1990 having a higher °Bx in 2011 than SS405 in 2012. Anthracnose differed by year, PD, N rate and variety where disease severity was highest in 2012, when sorghum followed sorghum, and summer weather was wetter than 2011. In both years, M81-E had less anthracnose damage than either forage sorghum with SS405 having a higher disease rating in 2012 than 1990 in 2011 due to rotation and drier summer in latter year. Higher °Bx and anthracnose ratings were seen at 88 than 22 kg N ha<sup>-1</sup>. Anthracnose ratings were lower at the April than June PD.

#### **Anthracnose impacts biomass and sugar yield of forage but not sweet sorghum**

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Impact of anthracnose on aboveground dry matter (ADM), estimated sugar yield (SY), and total fermentable sugar concentration (°Bx) of energy feedstock forage and sweet sorghum is undefined. A factorial study with irrigated SS405 forage sorghum and Topper 76-6 (2014) or M81-E (2015) sweet sorghums as whole plots, and 1, 2, 3, 5, 6, or 7 (2014 only) broadcast applications of Headline (12 fl oz A<sup>-1</sup>) at 28, 35, 42, 49, 56, 64, and 72 DAP [days after planting] as the split-plot treatment was used. A non-fungicide treated control was included. Anthracnose intensity was recorded at 7 to 14 d intervals from 30 DAP until harvest at soft dough; areas under the disease progress curves (AUDPC) for anthracnose were calculated from these ratings. On SS405, anthracnose intensified from onset at 30 to 45 DAP through harvest at 111 DAP when > 75% leaf area loss was noted for all but the 6- or 7- application programs. While Topper 76-6 was anthracnose-free, this disease intensified on M81-E from onset at 90 DAP until harvest at 133 DAP when > 75% leaf area loss was observed for all Headline programs and the non-fungicide treated control. In 2015, anthracnose AUDPC increased as Headline application number declined on SS405 but not M81-E. AUDPC was regressed against ADM, SY, and °Bx. In both years, ADM, SY, and °Bx declined by up to 30% as anthracnose intensified on SS405. Headline application number did not impact ADM, SY, and °Bx of the disease-free Topper 76-6.

#### **Managing Rhizoctonia crown and root rot of sugar beets utilizing azoxystrobin-alternative fungicides in Nebraska**

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In Nebraska, Rhizoctonia root and crown rot, caused by *Rhizoctonia solani*, is generally considered to be the most commonly occurring and damaging sugar beet disease. Previous studies have demonstrated that applications of azoxystrobin will effectively reduce disease and improve yield parameters. However, the almost exclusive use of Quadris (azoxystrobin) for more than a decade raises concern for resistance development by the pathogen to this fungicide. Studies were conducted from 2013-2015 to evaluate the performance of alternative fungicides currently registered for this disease in sugar beets (Priaxor, Proline, Headline, and Vertisan) and compare their performance to Quadris. The study consisted of 16 treatments: 1) untreated control, 2-6) applications applied in-furrow at planting, 6-10) foliar applications after 4 inch soil temperatures averaged 65° F for three consecutive days, and 11-15) both in-furrow and foliar applications. Data collected included multiple disease counts during the season, and sucrose and root yield determinations at harvest. Our results indicated that Quadris, Priaxor, and Proline all performed similarly, significantly reducing disease incidence and increasing sugar yields compared with untreated controls. We further determined that better results were obtained with the combination of in-furrow and foliar applications later in the season than either treatment alone.

#### **Monitoring pomegranate pathogens towards developing effective disease management program**

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Pomegranate (*Punica granatum* L.) is becoming a popular alternative crop in the Southeastern United States due to its nutritional benefit and emerging production potential in the area. Studies were conducted in 2015 to monitor pathogens prevalence from bud break to fruit maturity. Buds were collected from late January through March from three locations in Florida, two in Georgia, and one in South Carolina and were divided into six different stages from an unopened flower bud to fruit initials. The first, second and third stage buds were cut into halves whereas fourth, fifth, and sixth stages were cut into six longitudinal sections and were individually plated in V8 agar media and incubated at 21°C. Plates were monitored daily and the pathogen was identified based on morphology. Three orchards in Central Florida were also monitored every other week from late February to August and samples were collected to monitor pathogen prevalence. Several pathogens were recovered in the absence of any symptoms during the bud stages. The first symptoms observed on fruits were anthracnose caused by *Colletotrichum* sp. With increasing precipitation, the disease spread leading to spotting and blighting of leaves, stems and fruits. Further disease development, including the blighting of shoots and fruit mummification became more prevalent towards the mid to late season and was caused by combination of several pathogens.

#### **A meta-analysis of efficacy of anaerobic soil disinfestation on soil borne pathogen suppression**

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Anaerobic soil disinfestation (ASD) is a widely applied pre-plant soil treatment practice to control soil borne pathogens under variable environmental conditions. A meta-analysis on multiple ASD studies (533 studies from 46 published/unpublished articles) on soil borne pathogens including bacterial, oomycete and fungal pathogens was conducted to determine the effect sizes and efficacy of ASD on pathogen control. We also examined various environmental conditions as moderator variables to explore the effectiveness of ASD against pathogens. Bacterial (*Ralstonia*) and oomycete (*Pythium*

and *Phytophthora*) pathogens were highly suppressed by ASD treatment (80%-88%), whereas across fungal pathogens (*Cylindrocarpon*, *Fusarium*, *Macrophomina*, *Rhizoctonia*, *Sclerotium*, *Verticillium*) suppression ranged from 59%-86%, with the exception of *Sclerotinia* (15%). Among different host specific *F. oxysporum* (*Fo*) pathogens (237 studies), spinach and tomato wilt caused by *Fo* f. sp. *spinaciae* (87%) and *Fo* f. sp. *lycopersici* (76%) were significantly suppressed by ASD. Under most environmental conditions, suppression of pathogen inoculum due to ASD treatment ranged from 62%-94%, that included study types, soil temperature, soil types and incubation period. Our meta-analysis indicates that ASD is effective against a broad range of soil borne pathogens and optimization to environmental conditions and cropping systems is likely to improve treatment consistency.

#### **Evaluation of the weather-based spray advisory program MELCAST, fungicide alternations and mulch to manage watermelon diseases in Missouri**

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Melons are economically important crops in Missouri. Each year, *Alternaria* leaf blight (ALB), anthracnose (ANT) and gummy stem blight (GSB) cause yield losses in melons. Most commercial growers use calendar-based fungicide spray schedule. MELCAST (MELon disease foreCASTer) is a weather-based disease-forecasting and spray advisory program that allows cantaloupe and watermelon growers to schedule sprays based on weather. From 2013–2015, there were 3-6 MELCAST sites in central, east and south east Missouri. Research plots were established at one of the sites in central Missouri at G. W. Carver Farm to examine MELCAST, mulches and fungicides in reducing diseases of watermelon using variety 'Crimson Sweet'. Fungicide sprays significantly ( $P < 0.05$ ) reduced diseases compared to non-sprayed plots. Alternating Bravo WeatherStik and Inspire Super or Quadris Opti and Luna Experience significantly reduced ALB, ANT and GSB. No significant difference was detected between these two plans. In 2014, total foliar biomass was significantly ( $P < 0.05$ ) higher with fungicide sprays (34.7 lb/plot) than without (9.1 lb/plot) for the plastic mulch plots. Disease suppression was not significantly different either between MELCAST and weekly sprays or between rye-vetch and plastic mulch. Use of MELCAST saved 1-2 fungicide sprays per season. Growers are encouraged to use mulch and to alternate fungicides in order to overcome risk of fungicide resistance and to improve productivity of watermelons.

#### **Temporal variation of soil biota in fumigated and non-fumigated potato fields**

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Soil biota plays a prominent role in the regulation of soilborne pathogen incidence and severity. Thus, microbial community structure is of particular interest in commercial potato production as fumigation practices have been shown to alter soil biota. Despite long-term use of fumigation in potato production, there is still a limited understanding of soil biota and pathogen recovery and regeneration. In this study, soil cores were taken from neighboring fumigated and non-fumigated potato fields located in five different states: Washington, Oregon, Idaho, North Dakota, and Minnesota. Each core was assessed for pathogen incidence and severity and changes in soil biota over a temporal scale. Fungal and bacterial soil microbial diversity was assessed using meta-barcoded primers on an Illumina MiSeq platform. Our results demonstrate that fumigation creates disparity between pathogen populations and other soil biota which causes disequilibrium when compared to non-fumigation. Future research may establish predictive models in evaluating pathogen incidence and severity as part of an integrated disease management system that seeks to improve sustainable soil health for high-quality potato production.

#### **Effect of temperature and sugar beet growth stage on yellowing decline caused by *Fusarium secorum***

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*Fusarium* yellowing decline caused by *Fusarium secorum* was recently reported in Minnesota. Conditions favorable for disease development were determined using three isolates (12-4P, 12-2P and 1189), three temperatures (55°C, 65°C, and 75°C), and three growth stages (3, 5 and 7 weeks old plants [WOP]). One *F. oxysporum* f. sp. *betae* isolate was used for comparison. Plants were inoculated using root dip ( $10^5$  microconidia or macroconidia  $ml^{-1}$ ) for two minutes. Evaluation for foliar symptoms was done every two days until harvest 35 days after inoculation, and area under disease progress curve (AUDPC) was calculated. Temperatures of 75 and 65°C were more favorable for disease development than 55°C. The mean AUDPC were 38, 29, and 4 for 12-4P, 12-2P and 1189 isolates, respectively. 12-4P isolate had significantly higher AUDPCs at 75 and 65°C for 3 WOP compared to both 5 and 7 WOP. 12-2P isolate resulted in no significant differences between AUDPCs at 3, 5, and 7 WOP within each temperature. However, 12-2P isolate caused significantly more disease at 75°C and 65°C compared to 55°C for 3 and 5 WOP. Isolate 1189 was least aggressive with no significant differences in AUDPC among all combinations of temperature and growth stage. Isolates 12-4P and 12-2P resulted in higher AUDPC than *F. oxysporum* f. sp. *betae* at the same temperature and sugar beet growth stage. Results suggest early planting in cool soils may help to manage the disease.

#### **Management of wilt diseases on tomato by organically acceptable methods**

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Soilborne wilt disease caused by fungal pathogen *Verticillium* spp, *Fusarium* spp and bacterial pathogen *Ralstonia solanacearum* often cause serious losses to tomato growers either by stunting or totally killing the plants prematurely. Rotation and chemical fumigation of soil is not feasible to many growers either due to limited farm size or organic growing conditions. Biofumigation and/or biologically based options for managing these pathogens are critical needs to these growers. A study was conducted with heirloom tomato 'Mortgage lifter' at the WVU Organic Farm to investigate the efficacy of bio-fumigants, biological antagonists, and resistant rootstock in managing wilt disease. Thirty five days after planting in contaminated soil, grafted (on resistant rootstock "Maxifort") tomato plants showed significantly higher plant vigor compared with non-treated check. All treatments had significantly lower symptomatic leaves than that of non-treated check at 35 days after planting. Cumulative harvests for six weeks showed that yield from all treatments except mustard cover crop were significantly ( $P < 0.001$ ) higher compared with non-treated check. Grafted plants produced 20 lb tomatoes/plant compared with only 11 lbs by non-treated check. Results suggest that organically acceptable methods can provide significant yield advantage to heirloom tomatoes in soilborne pathogen infested soil.

#### **Cryotherapy and Mini-plant Biological Indexing: New Tools for use in Citrus Clean Stock/Certification Programs**

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Shoot tip grafting (STG) followed by biological indexing using 10-14 month old indicator plants has been the mainstay for eliminating graft transmissible pathogens (GTP) from citrus accessions. We report the application of cryotherapy (freezing vegetative buds in liquid nitrogen followed by grafting onto seedling rootstocks) as an alternate, effective method for the elimination of certain GTPs. Because larger vegetative buds are used for cryotherapy, up to 1.5 mm, as compared to 0.1 mm of apical meristem buds used for STG, personnel become proficient after 1-2 weeks of training as compared to 6-12 months training needed for STG. We also have developed a biological indexing method using very young (mini) indicator plants, 60-90 days old. Side by side comparisons of 30 accessions using traditional and mini-plant biological indexing indicate the same level of sensitivity between



the two methods. The use of mini-plants for biological indexing requires much less time and greenhouse space, also GTP requiring cooler temperatures to produce symptoms may be indexed year-round by placing the mini-plants near the cooling pads in summer. The use of cryotherapy and mini-plant biological indexing will enable citrus clean stock and/or certification programs to be more productive while using fewer resources.

#### **Bacillus mycoides isolate J (BmJ): Plant protection through Systemic Acquired Resistance. A new option for plant disease management**

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*Bacillus mycoides* isolate J (BmJ) induced resistance provides control of bacterial, fungal, oomycete and viral pathogens on a wide range of crop plants. BmJ induced resistance is signaled through the NPR-1 gene and involves several PR proteins and ethylene. Optimal systemic resistance induction occurs 3-5 days after application of live cells and provides disease control for 14-21 days depending on the plant induced. BmJ is compatible with pesticides including; triazole, EDBC, and QoI class fungicides and a wide range of insecticides. Disease control equal to commercial standards has been achieved with BmJ alone or in combination with fungicides where BmJ replaces half the fungicide used in fungicide alone programs. BmJ has also been demonstrated to be of benefit to triazole and QoI fungicide resistance management programs in *Cercospora* leaf spot of sugarbeet control programs. BmJ used in combination with insecticides and rouging has produced >50% control of potato PVY in multiple years in the field. Research results also support disease control in cucurbit crops, tomato, pepper, potato, pecans, spinach, lettuce and sugarbeet with comparisons to commercial pesticide standards. BmJ is licensed to CERTIS USA by Montana State University and will be sold as a WDG formulation. Application rates are based on  $1 \times 10^7$  cfu/ml (60-240 gm/A depending on spray volume). Registration is expected with Canada's Pest Management Registration Authority (PMRA), the USEPA.

#### **Comparison of carbon quantity in anaerobic soil disinfestation**

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Anaerobic soil disinfestation (ASD) is a pre-plant alternative to chemical fumigation, used to manage soil plant pathogens, nematodes, and weeds. Compared to chemical fumigation, ASD was as effective for controlling these pests, and fruit yield often exceeded. Application of ASD in Florida includes incorporating composted broiler litter, 22 Mg ha<sup>-1</sup>, and molasses, 13.9 m<sup>3</sup> ha<sup>-1</sup>, tarping the soil, and saturating to capacity. The environment under the tarp becomes anaerobic during the treatment, allowing anaerobic microbes, which contribute to pathogen management, to thrive. The objective of this study was to compare various rates of molasses, 0-4 times the standard rate (SR), in greenhouse conditions. Treatments were compared using the following variables: soil conditions, plant vigor, soil microbiome, and weed count. Soil microbiome was investigated by extracting microbial DNA from the soil, taken periodically during the treatment, and using length-heterogeneity PCR. Shifts in the microbiome correlated to changes in soil pH and reduction potential. The microbiome fluctuated slightly in the no molasses treatment, while the microbiome of the SR changed throughout the treatment, and the pH temporarily decreased. Bacterial diversity decreased 7 day post treatment (dpt) for the treatments with molasses rates greater than the SR. The soil pH for these treatments dropped from pretreatment levels and remained acidic; pH for 4 times the SR was 1 dpt = 6.9 and 21 dpt = 5.3.

#### **Practical resistance to mfenoxam in *Pythium aphanidermatum* and its impact on managing *Pythium* root rot in poinsettia cultivars**

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Mefenoxam (mef) resistance is widespread among populations of *Pythium* in NC greenhouses, but resistant (MR) and sensitive (MS) isolates often are found concurrently. The combined utility of host resistance and mef to manage root rot in plants exposed to mixed populations of *P. aphanidermatum* was tested on seven poinsettia cultivars (cvs). One MR and one MS isolate were used, giving four inoculum/fungicide combinations: MR+mef, MR-mef, MS+mef, MS-mef. Plants were treated twice with the labeled rate of Subdue Maxx. Plants were scored for root rot (RR, 1 = 0% rot; 5 = 100%) and aboveground disease severity (DS, 1 = healthy; 5 = dead plant) 10 wk after inoculation. MS-mef treated plants had the highest RR (mean = 4.0) and DS (mean = 3.9) in all cvs. Mef controlled RR in all cvs inoculated with the MS isolate (mean = 1.6) but not in 5 of 7 cvs inoculated with the MR isolate (mean = 3.4), demonstrating practical resistance to mef in *P. aphanidermatum*. MR-mef treated plants had lower DS severity than MS-mef treated plants (3.3 vs. 3.9), suggesting that the MS isolate was more aggressive than the MR isolate. Treatment with mef lowered DS in MR inoculated plants only slightly (mean = 2.6). If confirmed with other MS and MR isolates, this may explain why MS isolates persist in facilities that regularly use mef. Resistant poinsettia cvs could be used to offset loss of fungicide efficacy and reduce losses to *Pythium* root rot in facilities with mixed *Pythium* populations.

#### **Evaluation of Commercial Hot and Bell Pepper Cultivars for Resistance to *Phytophthora capsici***

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*Phytophthora* blight (caused by *Phytophthora capsici*) is an important disease with a broad host range including Solanaceous, Cucurbitaceous, and Fabaceous crops. In pepper, *P. capsici* causes crown, root, and fruit rot as well as foliar lesions. Disease management relies primarily on the application of fungicides, planting of tolerant cultivars, and cultural practices. Field trials were conducted over the summers of 2015 and 2016 to evaluate 32 commercial hot and bell pepper cultivars in North Carolina (NC). Cultivars Martha-R and Meeting were found to be the most resistant to a group of isolates from NC. Ebano-R, Revolution, Paladin, and Vanguard showed intermediate levels of resistance. Bastille, Red Knight, and Plato were highly susceptible to *P. capsici*. Greenhouse experiments were conducted to determine the virulence of three individual *P. capsici* isolates across 48 commercial hot and bell pepper cultivars. The isolates exhibited different levels of virulence to the pepper cultivars screened for crown and root rot resistance. Cultivars CM334, Martha-R, Meeting, and Intruder were resistant to the isolates tested. In addition, we phenotypically characterized a *P. capsici* isolate collection from NC for fungicide sensitivity and mating type. This information will be useful for NC growers in selecting resistant cultivars and for future population genetics analyses.

#### **Managing Potato Pink Rot Using Chemical and Non-Chemical Strategies**

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Pink rot (*Phytophthora erythroseptica*) is a widespread disease on potatoes (*Solanum tuberosum*). Controlling pink rot becomes a challenge owing to the fast development of a fungicide-resistant *P. erythroseptica* population. To determine effective strategies for pink rot control, field trials were conducted in 2014 and 2015 using fungicides or crop rotation. Pink-rot-susceptible seed tubers (cv. 'Russet Norkotah') were used. *Phytophthora erythroseptica* inoculum was distributed in-furrow at planting prior to soil treatments. In fungicide trials, chemical and biological products were applied in-furrow at planting or through soil drenching after plant emergence. The crop rotation trial was conducted with "rotation crop-potato" sequences. Tuber yield was measured and disease severity was rated based on a "0 (healthy tuber) to 5 (>50% rot area)" scale at harvest. Fluopicolide, mfenoxam-oxathiapiprolin combinations, and fluopicolide/oxathiapiprolin plus *Bacillus* combinations significantly reduced pink rot. None of the tested biocontrol agents significantly reduced pink rot alone. Mefenoxam was effective when there was no mfenoxam-resistant population of *P. erythroseptica*. Oats, clover, and barley underseeded with ryegrass lowered pink rot severity levels, although it was not statistically significant. Soil microbial communities in rotation plots are being investigated using next generation sequencing technology.

### **The Nærstad model for potato late blight is available on the open source license technology platform VIPS**

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The Nærstad model for potato late blight (*Phytophthora infestans*) is a weather driven forecast model based on validation trials with spore traps and trap plants under Norwegian field conditions. The model combines sub models for spore production, release, survival, and infection, with calculations derived from hourly values of temperature, relative humidity, global radiation, leaf wetness (LW), and precipitation. LW may also be calculated with additional wind and net radiation parameters. The model output is presented as hourly risk values for infection of *P. infestans*. The Nærstad model is currently provided to users in Norway, Sweden and Latvia through the open source licensed technology platform VIPS ([www.vips-landbruk.no](http://www.vips-landbruk.no)). Data from most online weather stations or public weather data networks can be implemented into the VIPS system, including virtual weather stations based on weather forecasts. In VIPS, models such as the Nærstad model can easily be tested and validated under local conditions. Model outputs from multiple weather stations can be displayed in a map to visualize regional differences, in addition to more detailed outputs for each location. The open source license allows users of VIPS to make adaptations and changes to accommodate local needs, test and validate models locally or as part of an international network, and eventually provide the models directly to end users through the same system.

### **Control of tulip mild mottle mosaic disease and tulip streak disease based on soil diagnostic**

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Tulip mild mottle mosaic disease, cause of *Tulip mild mottle mosaic virus*, TMMMV and Tulip streak disease, cause of Tulip streak virus, TuSV transmitted by *Olpidium virulentus* cause chronic damage and become one of the factors of avolition on produce of tulip bulb in Toyama, Japan. The management of both viral diseases depends on use of fungicide, a fertilizing efficiency-control type fertilizer and cultivation of late time. However, it is necessary to choose the appropriate control measures according to the level of both viral disease occurrence risks on farmer's field. In this study, we developed management of both viral disease consisting of risk evaluation of both viral diseases on tulip field by the quantification of TMMMV and TuSV from soil using real-time PCR, evaluation of individual control measures for both viral diseases by meta-analysis and selection of appropriate control measures according to the level of both viral diseases occurrence risks on tulip field.

### **Management of Tomato chlorotic spot virus, an emerging tospovirus of tomato causing severe losses in south Florida**

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*Tomato chlorotic spot virus* (TCSV) is a devastating tospovirus transmitted by thrips. TCSV was first reported in the USA from tomato plants in Homestead, FL in 2012. Outbreaks of TCSV in Homestead area since 2014 have caused significant losses to growers. TCSV has become established in south Florida and it occurs more widely with greater frequency across this region, bringing a serious concern that TCSV may spread to other tomato production regions. Field trials have been conducted in Homestead since 2015 to develop management strategies for TCSV. Fourteen tomato varieties resistant to *Tomato spotted wilt virus* (TSWV) were evaluated. In the spring trial in 2015, all tested varieties exhibited varying levels of resistance to TCSV (10-40% vs 100% infection in the commercial standard cv. 'FL 47') two months after transplanting. In the two repeated trials carried out in the fall of 2015, all these varieties had lower than 8% disease incidence 16 weeks after transplanting, whereas 50% plants of cv. 'FL 47' showed TCSV symptoms. In an insecticide trial conducted in the spring of 2015, only spinetoram (Radiant®) and cyantraniliprole (Exirel®) significantly ( $P < 0.05$ ) reduced TCSV incidence compared to the untreated control. In the fall trials of 2015, plant activator acibenzolar-S-methyl (Actigard®) numerically reduced TCSV incidence. The use of metalized UV-reflective plastic mulch failed to suppress the disease. Results from this study will be reported and discussed in detail.

### **Variation within and among laboratories in detection of *Candidatus Liberibacter asiaticus* using qPCR**

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Huanglongbing (HLB) disease has ravaged the Florida citrus industry during the last decade and poses a serious threat to citrus production worldwide. *Candidatus Liberibacter asiaticus* (CLAs) is the presumed causal agent of HLB in the United States. Diagnosis of CLAs infection is based on PCR; qPCR for wide-scale surveys and conventional PCR for confirmation. The objective of our research was to compare results among multiple laboratories using slightly different qPCR protocols to confirm the sensitivity and diagnostic reliability of qPCR. An end point dilution series of CLAs-positive nucleic acid was prepared and distributed to 4 laboratories in which CLAs diagnostics are routinely performed. In each laboratory qPCR diagnostics were performed using the dilution series as template, however, specific protocols typical for each laboratory varied slightly. Results were compared within and between laboratories to determine linearity, amplification efficiency, end point and consistency. Excellent agreement in results was seen among protocols within individual laboratories as well as between individual laboratories. Regression analysis indicated that regardless of protocol, PCR efficiencies were between 95 and 105%. In addition, results confirmed a Ct value of 38 for the endpoint (i.e. a single copy of target). Our results support the robustness and diagnostic reliability of the currently approved qPCR protocol.

### **Regulatory implications of a newly discovered transmission mechanism for the pathogens that cause huanglongbing (citrus greening disease)**

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Huanglongbing (HLB, citrus greening disease) is one of the most devastating citrus diseases in the world. In Florida, it is associated with a bacterium *Candidatus Liberibacter asiaticus* (Las) and transmitted by a psyllid, *Diaphorina citri* Kuwayama. Lee et al. (2015; PNAS 112: 7605–7610) documented a new transmission mechanism for Las. An adult female psyllid, or her mate, infects the new growth where she lays eggs. The localized infection becomes a source for developing nymphs. Progeny can repeat the process as soon as they become adults. Since each female can lay 750 or more eggs, and psyllids can complete development in 15 days, the potential for increase of infected insects is rapid. In the laboratory, plants colonized by infected psyllids usually develop disease, but symptoms may be delayed for months or years. Catastrophic spread of Las can occur long before symptoms manifest. This delayed expression of disease symptoms could allow for the disease to spread undetected.

### **Known unknowns and unknown unknowns: Fungi intercepted at the U.S. borders**

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Global trade in plants and plant products continues to increase yearly and has the potential to move fungi associated with poorly studied hosts around the world. USDA Animal and Plant Health Inspection Service (APHIS) plant pathology identifiers at ports across the U.S. are responsible for identification

of fungi on products arriving by sea, land and air. In 2015, over 18,000 interceptions were examined by USDA APHIS plant pathology identifiers. These interceptions covered about 600 genera of hosts, with more than 250 genera of fungi identified on these hosts. The inspected hosts comprise ornamental, forestry, and horticultural plants and plant products. Nearly one quarter of the interceptions were from cargo awaiting entry decisions and required identifications in less than 48 hours. The rest were from plants and plant products seized from passenger baggage, mail, ship stores and other sources and represent some of the as yet unknown fungal/host combinations that could be arriving in the U.S. via these pathways. If a fungus not known to be present in the U.S. is intercepted on a shipment, that shipment is either destroyed or re-exported, which underscores the need to know which fungi have been reported in the US and also the need for systematic research in widespread fungal groups. An overview of intercepted fungi will be presented including fungi intercepted for the first time from various ports and not known to occur in the U.S.

#### **Functional genomic analysis of *Botrytis cinerea* isolates from Ohio**

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*Botrytis cinerea* is one of the most widely spread phytopathogenic fungi displaying a broad host-range. While *Botrytis* is generally considered a model necrotroph, systemic symptomless infections have also been reported. Previous studies have shown significant genomic variation between gray and rare bikaverin producing pink isolates, but their contribution to diversity and host/pathogen relationships is understudied. We aim to elucidate genomic features of symptomless and host specific infections, and biochemically characterize virulence determinants. For this, we sequenced the genomes of 4 isolates from Ohio: 1 gray, isolated as asymptomatic endophyte of apple blossoms; 1 pink and 1 gray, isolated from infected flowers of *Matthiola incana*; and 1 purple, obtained from distinct sectors of pink isolate. We compared all 4 genomes to B05.10 and T4 reference genomes. Results showed large polymorphisms within coding regions of predicted genes (52,710, missense; 418 nonsense and 80,430 silent) and deletion of gene clusters (GC) associated with virulence. In both pink and purple isolates the botcinic acid GC was deleted, while in the gray asymptomatic isolate the abscisic acid GC was deleted. Investigation of virulence determinants among isolates showed a fully functional sclerotia formation, while the pink and purple strains alone were impaired in oxalic acid formation. Our results suggest that genetic diversity plays a significant role in species complexity and lifestyle.

#### **Virus testing in certified strawberry nursery plants in Oregon**

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*Strawberry crinkle virus* (SCV), *Strawberry mottle virus* (SMoV), *Strawberry mild yellow edge virus* (SMYEV), and *Strawberry vein banding virus* (SVBV) are aphid-borne strawberry viruses frequently found in the Pacific Northwest. As such, they are excellent indicators for the effectiveness of nursery certification programs in maintaining virus free plants. Oregon nurseries import certified strawberry plants primarily from California and multiply these plants prior to sale to the final consumer. Testing for viruses in certified plants reveals the virus status of the plants and measures the effectiveness of the originating state's certification program. A survey for these four viruses was performed in a Klamath County nursery multiplying certified strawberry plants. A total of 59 plant samples were collected and tested for SCV and SMYEV while 118 samples were tested for SMoV and SVBV. SVBV was detected using PCR with SVBV-specific primers. SCV, SMoV, and SMYEV were not detected using RT-PCR with virus-specific primers. SVBV was detected in one out of 118 samples tested. These results demonstrate the continued need for virus testing of strawberries throughout the plants' entire life cycle in an official certification program.

#### **National Seed Health Accreditation Pilot Program: Monitoring seed health of seeds imported into the United States**

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The risk of introducing plant pathogens into new areas by seed movement is a critical issue in agriculture. Part of the mission of USDA-APHIS is to protect U.S. agriculture from foreign pathogens. In order to help fulfill this mission, a pilot program, the U.S. National Seed Health Accreditation Pilot Program (NSHAPP), has been authorized by USDA-APHIS. NSHAPP is a voluntary program for seed importers that is administered by the National Seed Health System at Iowa State University Seed Science Center in Ames, IA. NSHAPP aims to prevent the introduction of plant pathogens into the United States by organizing the testing of imported seed. The scope of program focuses on cucumber green mottle mosaic virus (CGMMV) in cucumber, melon and watermelon seed. CGMMV causes severe disease of cucurbit crops, and was first detected in the U.S. in 2013 in California field production. Testing for CGMMV under NSHAPP is done by an ELISA method validated by the Int. Seed Testing Assoc. Participants report data on tested seedlots monthly, but positive seed tests must be reported immediately. Positive seedlots must be destroyed or re-exported. This program represents a proactive approach to reducing the risk of pathogen introduction in imported seed and demonstrates effective collaboration between seed importers and USDA-APHIS. Successful implementation of the pilot program may lead to expansion of voluntary testing and reporting to include other pathogens in imported seed.

#### **Assessing Goss's bacterial wilt and leaf blight of corn in the High Plains of Texas with infrared aerial imagery**

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Outbreaks of Goss's bacterial wilt and leaf blight of corn, caused by *Clavibacter michiganensis* subsp. *nebraskensis*, were reported in the Texas High Plains from 2013 to 2015. The disease was identified based on symptomology, bacterial streaming, colony characteristics and ImmunoStrip test results. Impact of the disease on corn is poorly understood in this region, thus the objective of this study was to evaluate disease development using aerial imagery. Fields were photographed from a fixed-wing aircraft from mid-July to late-September of each year. Images were effective at differentiating resistant and susceptible hybrids and improved scouting efficiency when assessing incidence and severity. Disease onset differed by location but initial infections were easily identifiable. In general, infection foci were non-random or aggregated; however, edge effects were observed in two areas adjacent to fields where the disease was observed the previous year and reduced tillage was implemented. Yield reductions ranging from 12 to 53% were observed in areas of some fields; whereas, other fields exhibiting >85% incidence were abandoned for grain production and cut for silage. These results indicate that Goss's wilt has the potential to negatively affect corn production under favorable conditions. Increased awareness through educational materials, including information on hybrid selection, crop rotation and residue management has allowed for better management of the disease.

#### **Understanding fungal reservoirs: Tracking *Aspergillus* populations in air, soil, crop debris and the reproductive organs of Bt and non-Bt corn**

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*Aspergillus flavus* can colonize a wide range of environments. The advent of effective biocontrol agents makes it important to know where fungi overwinter, because the amount and toxigenicity in each reservoir may determine the most effective site(s) to apply biocontrol. *Aspergillus* propagules on leaf, husk, silk and tassel of Bt and non-Bt corn, and from soil and air/dust were quantified using dilution plating on dichloronitroaniline rose Bengal agar. There was no effect of Bt corn on the amount of *Aspergillus* propagules. *Aspergillus* propagules in air/dust varied dramatically from year to year (0-278 cfu/1000L). We studied corn hulls, cob and kernel fragments that accumulated near on-farm grain storage bins in normal farm operations and were

periodically moved to the edge of an adjacent field and disked into soil in fall tillage. The following March, *A. flavus* propagules in soil samples ~20, ~30 and ~40 meters from the bins were 1,500-28,000 CFU/g, with ~3 times as many at 20m as at 40m, but 24% aflatoxigenic at all distances. In January 2016 similar debris and dust accumulating near on-farm grain storage bins contained 14,500±5,400 CFU/g *A. flavus* propagules. Additional studies on *A. flavus* propagules from rice, sesame and Illinois corn field soil and plant debris will be discussed. The findings are consistent with a substantial plant debris-associated soil populations. These findings are very useful in selecting optimal biological control strategies for aflatoxin.

#### **Impact of stalk rot diseases on sweet sorghum sugar concentration and content**

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The sugar-rich nature of sweet sorghum [*Sorghum bicolor* (L.) Moench] makes it an attractive bioethanol feedstock. Fusarium stalk rot (*Fusarium thapsinum*, FT) and charcoal rot (*Macrophomina phaseolina*, MP) are high priority fungal diseases of sorghum, which can potentially affect sweet sorghum biofuel traits. The objective of this study was to test the effects of FT and MP on sweet sorghum sugar concentration and content. Seven parental lines and 12 hybrids were field evaluated in 2014 and 2015. At 14 d after flowering, plants were inoculated with FT, MP, and phosphate-buffered saline (control). Plants were harvested at 35 d after inoculation and measured for disease severity using stem lesion length. Juice volume (per plant) extracted from stalks was measured and subjected to HPLC to determine sugar concentration (mg/mL). ANOVA revealed non-significant pathogen main or simple effects on glucose and fructose concentration and significant genotype × pathogen effect on sucrose concentration. Pathogens did not significantly affect the total sugar concentration (= ∑ sucrose, glucose, fructose). However, when analyzed for content (g/plant), genotype × pathogen and environment × pathogen effects were evident for total sugars and sucrose. Pathogens affected glucose and fructose content in an environment-specific manner. Therefore, in general, stalk rot pathogens do not affect the concentration of major sweet sorghum sugars, but do affect sugar content.

#### **Effect of planting date and peanut cultivar on leaf spot epidemics and pod yield**

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Planting date can affect the risk of losses to early and late leaf spot caused by, *Cercospora arachidicola* and *Cercosporidium personatum*, respectively, of peanut, *Arachis hypogaea*, in both conventional and organic systems. The objective of this study was to characterize the effect of planting date on leaf spot epidemics and yield in new cultivars with moderate tolerance to these diseases. A field trial was conducted in 2015 in Tifton, GA. Treatments were six planting dates (24 and 27 April, 4, 11, 19, and 26 May) arranged factorially with two cultivars, Georgia-06G and Georgia-12Y. Experimental design was a randomized complete block design with 4 replications. No foliar fungicides were applied. Late leaf spot was the predominant disease. Epidemics were severe in plots planted at the later dates. Final leaf spot ratings (Florida 1-10 scale) and AUDPC increased linearly with later planting date (Julian day) for both cultivars. Yield of Georgia-06G decreased linearly, and yield of Georgia-12Y decreased according to a quadratic function with later planting. For most planting dates, final leaf spot severity and AUDPC were lower, and yield was higher for Georgia-12Y than for Georgia-06G. The combination of early planting with Georgia-12Y shows potential for reducing risks of losses, leaf spot, and maximizing yield in situations such as organic production where fungicide use would be minimal.

#### **Fungal profiles in ginger root and cayenne pepper powders from U.S. retail**

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A limited survey was conducted to determine the mycological quality (mould and yeast levels and profiles) of ginger and cayenne pepper powders sold in local stores in the Washington, D.C. area. Ginger powder samples were purchased from four companies (A, B, C, and D), while cayenne pepper was obtained from two companies (A and B). Powders packed in capsules as well as in bulk bottles and jars were examined. All ginger samples tested during this study contained live fungi. Mould levels ranged between  $2.4 \times 10^3$  and  $6.2 \times 10^4$  colony forming units/g (cfu/g). The most common species were *Aspergillus flavus* and *Aspergillus niger* followed by eurotia and *Fusarium* spp. No yeasts were found in any of the samples tested. Some variation in fungal species and contamination levels were apparent among different companies. Higher levels of fungal infestation were observed in cayenne pepper samples. Seventy one percent of the analyzed samples were carrying live moulds while only one sample tested positive for yeasts. Mould counts ranged between <100 and  $6.9 \times 10^5$  cfu/g. The most frequent fungal contaminants were *A. niger*, *A. flavus*, and eurotia (*E. chevalieri* and *E. rubrum*) followed by *Aspergillus* spp. and members of the Mucorales family. Variation in mould levels existed between the two companies and among lots of the same company.

#### **Wheat streak mosaic virus differentially affects soft red winter wheat cultivars**

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Wheat viruses are known to impact production throughout the world. A statewide survey conducted in 2012 identified several viruses in wheat samples taken from fourteen Ohio counties. Wheat streak mosaic virus (WSMV) was identified in samples from two counties, Auglaize and Champaign. WSMV is a positive sense, single-stranded RNA virus transmitted by wheat curl mites (*Aceria tosichella*), and is known to cause severe yield loss and stunting in hard red winter wheat. However, there is little information available about WSMV specific to soft red winter wheat cultivars grown in Ohio. To assess its potential impact, nineteen Ohio cultivars were mechanically inoculated with WSMV at Feekes stage 1. All cultivars supported systemic infection. Over 21 days post-inoculation, individual plants were rated for symptom severity, final height, and final wet weight. Across cultivars, inoculated plants exhibited an average 13.2% decrease in plant height, and a 32.1% decrease in wet weight. The cultivars differed in virus responses, as determined by generalized linear mixed model analysis. This experiment demonstrates that WSMV infection affects soft red winter wheat cultivars differentially, which may lead to the identification of cultivars that minimize yield loss.

#### **Occurrence of a Citrus canker strain of limited host specificity in Texas**

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In October 2015, a Mexican lime exhibiting citrus canker-like symptoms was found by the USDA-APHIS-PPQ citrus disease survey in a residential property in Cameron County, Texas. The Texas A&M University-Kingsville Citrus Center detected citrus canker using a real-time PCR analysis. The USDA-APHIS-PPQ Lab in Beltsville, MD confirmed that it was a *Xanthomonas citri* subsp. *citri* isolate. A delimiting survey was initiated by USDA and by February 2016, 169 suspect leaf samples were sent to the PPQ Beltsville lab for analysis. Of 118 samples positive for citrus canker, all within a 5-mile radius, 116 were from Mexican lime, 1 was from Kaffir lime and 1 from Ponderosa lemon. Survey data collected on stem lesions suggested the oldest lesions to be between 4 – 6 years old. To examine the host range of the TX citrus canker strain, four pots each of 11 different citrus varieties, including the major commercial varieties grown in TX, were inoculated by syringe infiltration of symptomatic crude leaf extracts. The only citrus plants

that developed clear canker lesions were Mexican limes. A combination of serological and molecular tests also suggested that the Texas citrus canker isolate was different from the typical Asiatic strain (Xac A) and more similar to other known *X. citri* subsp. *citri* strains with limited host range such as Xac A<sup>W</sup> or A\*. Xac A<sup>W</sup> strain was previously reported from Florida on Mexican lime and Alemow, a close relative of Kaffir lime.

#### **A new 'Candidatus Phytoplasma pini'-related strain associated with witches'-broom of Pinus spp. is detected in Maryland**

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Phytoplasmas are insect transmitted specialized bacteria that cause devastating diseases in crops and natural ecosystems. Pine trees (*Pinus* spp.) showing abnormal shoot branching and witches' broom symptoms in a wooded area of Laurel, Maryland, were examined for the presence of phytoplasmas. Nested PCR assays for amplification of the 16S ribosomal RNA gene (rDNA) sequences were used on pine tree DNA samples to confirm possible association of a phytoplasma with the disease. Comparison of restriction fragment length polymorphism (RFLP) patterns and nucleotide sequence analysis of 16S rRNA gene fragments revealed that several trees were infected by a 'Candidatus Phytoplasma pini'-related strain (MDPP). The 16S rDNA sequence of MDPP shares 98.4% similarity with that of the 'Candidatus Phytoplasma pini' reference strain reported from Spain (isolate P127). The collective 16Sr DNA F2nR2 RFLP pattern is distinct from the reference patterns of all previously delineated subgroups, and is most similar to that of subgroup 16SrXXI-A with a pattern similarity coefficient of 0.79. Since the similarity coefficient value is lower than the threshold (0.97) for new subgroup recognition, MDPP may represent a new subgroup within the group 16SrXXI. The new subgroup is hereby designated as 16SrXXI-B. To our knowledge, this is the first report of a 'Ca. Phytoplasma pini'-related strain associated with pine trees in North America and possibly the entire western hemisphere.

#### **The phytoplasma associated with purple woodnettle witches'-broom disease in Taiwan represents a new subgroup of the aster yellows phytoplasma group**

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In October 2013, a new disease affecting purple woodnettle (*Oreocnide pedunculata*) plants was found in Miaoli County, Taiwan. Diseased plants exhibited leaf yellowing and witches'-broom symptoms. Molecular diagnostic tools and electron microscopic cell observation were used to investigate the possible cause of the disease with a specific focus on phytoplasmas. The result of PCR with universal primer pairs indicated that phytoplasmas were strongly associated with the symptomatic purple woodnettles. The virtual restriction fragment length polymorphism (RFLP) patterns and phylogenetic analysis based on 16S rDNA and ribosomal protein, *rplV-rpsC* region revealed that purple woodnettle witches'-broom phytoplasma (PWWB) belongs to a new subgroup of 16SrI and rplI group and was designated as 16SrI-AH and rplI-Q, respectively, herein. RFLP analysis based on *tuf* gene region revealed that the PWWB belongs to tufI-B, but phylogenetic analysis suggested that PWWB should be delineated to a new subgroup under the tufI group. Taken together, our analyses based on 16S rRNA and *rplV-rpsC* region gave a finer differentiation while classifying the subgroup of aster yellows group phytoplasmas. To our knowledge, this is the first report of a 'Candidatus Phytoplasma asteris'-related strain in 16SrI-AH, rplI-Q and tufI-B subgroup affecting purple woodnettle, and of an official documentation of purple woodnettle as being a new host of phytoplasmas.

#### **Three races of *Xanthomonas campestris* pv. *vitians* causing bacterial leaf spot on lettuce identified**

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*Xanthomonas campestris* pv. *vitians* (Xcv), the causal agent of bacterial leaf spot on lettuce, reduces lettuce quality and yield worldwide. Although strain specific, host resistance is the most feasible method to reduce losses. Therefore, we screened lettuce germplasm for resistance using the broadest diversity within the pathogen population. A collection of 120 strains were assigned to one of six sequence types (A, B1, B2, C, D and E) by multilocus sequence typing (MLST) using concatenated sequences of *rpoD*, *dnaK*, *fyuA*, *gyrB*, and *gapI*. The abaxial sides of lettuce leaves were infiltrated with single bacterial isolates ( $1 \times 10^8$  CFU/ml) representing each sequence type. Tissue collapse beginning at 30 hr and resulting in light-brown papery lesions at 48 hr was considered an incompatible reaction. Replicated assays with strains were conducted with germplasm for which resistance was identified. This method was used to determine that the single dominant gene, named *Xanthomonas resistance 1* (*Xar1*), confers complete resistance to MLSTs B, D, and E. Subsequently, germplasm was identified with complete resistance to strains from sequence type A and a separate source of resistance was identified to strains from sequence type C. The genetics of resistance to sequence types A and C is being elucidated and we are completing analysis of all strains on resistant germplasm. However, the data gathered to-date indicate that there are at least three races of Xcv.

#### **Candidatus Liberibacter solanacearum associated to *Physalis philadelphica*, a new solanaceous host**

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*Candidatus Liberibacter solanacearum* (CaLs) bacterium is an economically important plant pathogen of solanaceous is vectored by the psyllid *Bactericera cockerelli*. Recently, were observed in husk tomato (*Physalis philadelphica*) fields in Toluca Valley in the state of Mexico, Mexico. Plants exhibiting stunting, severe leaf chlorosis, shortened internodes, curling of leaves, aborted flowers, deformation and reduced fruit size were observed, affecting the productive period. Large numbers of the psyllid *B. cockerelli* were observed on the crop. The objective of this research was to detect CaLs in husk tomato plants with similar symptoms as described above and whether this bacterium was present in *B. cockerelli* populations in the field. In 2015, we collected 250 samples from symptomatic plants and five psyllid *B. cockerelli* per plant in commercial plots in Toluca Valley. The molecular detection of CaLs was performed using OA2/OI2c, CLipoF/OI2c and Lp Frag 1-25F/427R primers. The amplified fragments were sequenced using Sanger methodology. Sequence alignment was carried out with the sequences belonging to the haplotypes currently recognized in GenBank database. The SNPs were identified visually. Haplotype B of CaLs was found in 100% of the plants tested, whereas in the insect found A and B haplotypes. This information will enable the approach of management strategies to husk tomato production.

#### **Use of an unmanned aerial vehicle to assess tomato spotted wilt severity in large peanut mapping populations**

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Recombinant inbred line (RIL) populations of peanut (*Arachis hypogaea* L.) are being used to develop markers for resistance to several diseases, including tomato spotted wilt, caused by Tomato spotted wilt virus (TSWV). In efforts to develop molecular markers to assist in selection for resistance to TSWV, several populations have been developed from parents with varying levels of TSWV resistance. Mapping programs are faced with the challenge of phenotyping large numbers of genotypes in a timely manner with a limited number of people. Through the use of an unmanned aerial vehicle, imaging techniques, and ground truthing, we developed an analysis method to quickly assess spotted wilt severity in the field using selected RILs from four mapping populations of peanut. With adequate ground truthing, spotted wilt is easily differentiated from other diseases in the field by the color and the visible stunting of the plant. By using a greenness equation in Matlab to identify yellow pixels we can rate the severity of the disease in

many small plots in a short amount of time. Both Matlab greenness values and severity values from APS Assess analysis of aerial images correlated well with field disease ratings. This technique allows for quick acquisition of field response data from large mapping populations.

#### **Reporting the occurrence of *Ralstonia* based wilts of chillies in major vegetable growing areas of Punjab**

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*Ralstonia solanacearum* (Rs), is one of the most severe pathogens of solanaceous crops with a very wide host range. Recently, a survey was conducted in districts of Punjab Province including Okara, Chiniot and Faisalabad to determine the incidence of bacterial wilt of chillies. The maximum disease incidence (58%) was recorded in district Okara followed by Chiniot (43%). The least disease incidence was found in district Faisalabad with 20% occurrence. The samples were collected and after surface sterilization, placed on casamino acid-peptone-glucose-agar (CPG) medium. The colonies obtained on CPG medium were transferred on 2,3,5-triphenyltetrazolium chloride (TZC) medium. It was observed that the colonies on TZC were irregular, mucoid and white with pink centers. Out of all the isolates tested, 72% showed positive hypersensitive reaction (HR) with variable response. Only 40% of these HR positive isolates were confirmed as *R. solanacearum* through simple staining, streaming, KOH solubility, biochemical, pathogenicity and Plate Trapped Antigen-Enzyme Linked Immunosorbent Assay (PTA-ELISA) tests. Molecular confirmation of *R. solanacearum* was also done through genomic DNA using Rs-specific primers.

#### **Identification of the causal agents of soft rot of potato in Bangladesh and activity of biocontrol agents against the pathogens**

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Potato is a staple food crop in Bangladesh. Bacterial soft rot is a major postharvest threat to potato, reducing yield approximately 37% in storage systems. Limited research has been conducted to identify and manage the causal agents of soft rot in Bangladesh. Eighteen bacterial isolates were collected from twelve potato-growing regions of Bangladesh in 2015. Sixteen of the isolates produced deep pits on Crystal-Violet Pectate (CVP) medium, were gram negative, oxidase negative, and hypersensitive response (HR) positive on tobacco, characteristic of *Pectobacterium*. Two isolates produced shallow pits on CVP medium, were gram negative, oxidase positive, fluorescent, and weakly HR positive, characteristic of *Pseudomonas*. All isolates caused soft rot on potato tubers. Partial 16s ribosomal DNA sequences from 16 presumptive *Pectobacterium* isolates exhibited >85% identity with *Pectobacterium* spp. sequences and two presumptive *Pseudomonas* isolates exhibited 90% identity with *P. fluorescens* and *P. putida* sequences previously deposited in GenBank. Fifteen *Pseudomonas* sp. strains with known biocontrol activity were tested for *in vitro* antibiosis against the soft rotting isolates. The biocontrol agents inhibited the growth of all *Pectobacterium* isolates but neither of the *Pseudomonas* isolates. Biological control of potato soft rot by *Pseudomonas* spp. is promising but additional research is needed to identify potential biocontrol agents for soft rotting *Pseudomonas* spp.

#### **Race characterization of *Pyrenophora tritici-repentis* population from Lithuania and Romania**

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Tan spot, caused by *Pyrenophora tritici-repentis* (*Ptr*) is an economically important disease of wheat. Eight races exist of this fungal population. In this study, 223 single spore isolates from Lithuania (n=114), and Romania (n=109) were evaluated for their race structure through genotypic and phenotypic characterization. Molecular variation was identified through PCR with *Ptr ToxA* and *Ptr ToxB* genes specific primer. Of these 223 isolates genotyped, 110 isolates were further phenotyped by inoculating them individually on two-week old seedlings of tan spot wheat differentials Glenlea, 6B365, 6B365, and Salamouni. Nearly 86% (191/223) isolates genotyped contained *ToxA* genes. Phenotypic characterizations on 110 isolates identified 69, 6, 13, and 4 isolates *Ptr* race 1, 2, 3, and 4, respectively. Eighteen of 223 isolates lacking in *Ptr ToxA* gene could not fit into currently identified 8 races as they produced necrosis on Glenlea and chlorosis on 6B365. These results indicate that a diverse *Ptr* population exists in Lithuania and Romania. This information would help the regional wheat breeding programs in the development of durable tan spot resistance cultivars.

#### **Morphological and molecular identification of *Phoma medicaginis* var. *medicaginis* causing spring black stem and leaf spot of alfalfa in Nevada**

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In June 2015, an outbreak of foliar blight disease was observed in an alfalfa (*Medicago sativa* L.) field with more than 50% of plants affected. Individual plants exhibited rapid death of foliage with sporadic or coalesced lesions on both lower leaves and stems. To isolate the causative agent, necrotic stem and leaf tissue, as well as pycnidia, were surface sterilized and then placed onto potato dextrose agar (PDA) amended with streptomycin sulfate and incubated at 22°C in the dark. Colonies of *Phoma* species were obtained from all samples collected from 5 separate field locations. Ten single spore cultures (SSCs) were obtained from 10 isolates and compared morphologically. All SSCs and isolates produced almost black colonies with abundant hyaline, oval to cylindrical, single-celled conidia with no significant morphological variation observed. A 540 bp DNA fragment was amplified from the ITS regions of rRNA genes and it was sequenced directly from both strands. Sequence analysis revealed that all 10 SSCs had the same amplicon sequence (Accession No. KU695570), with 100% homology to the corresponding regions of *P. medicaginis* var. *medicaginis* (Accession No. AY504634). Thus, the pathogen isolated from diseased alfalfa plants in Nevada was determined to be *P. medicaginis* var. *medicaginis*, the only pathogen associated with the outbreak. This is the first confirmed outbreak of spring black stem disease in Nevada since 1977 when it was initially reported in the state.

#### **Identification of a new *Mastrevirus* of *Saccharum barberi*, *Saccharum officinarum* and *Saccharum spontaneum* in Florida**

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A metagenomic study was conducted to identify viruses present in 180 leaf samples of *Saccharum* species collected in 2013/2014 from plants in commercial fields in the Everglades Agricultural Area and the germplasm collection of the USDA-ARS Sugarcane Field station at Canal Point, FL. The objective of this study was to identify known and potentially new sugarcane viruses. In addition to previously reported sugarcane viruses in Florida, we identified the presence of a potential new *Mastrevirus* species. Since there are no *Mastrevirus* species reported from Florida, and viruses in this genus such as *Sugarcane streak virus* and *Maize streak virus* are known to cause diseases in sugarcane, efforts were made to obtain full length viral genome sequences from individual plants of *Saccharum* spp. Rolling circle amplification followed by enzymatic restriction and cloning was used to obtain nine full genome length sequences (each approximately 2.8 kb): three from *S. barberi*, two from *S. officinarum* and four from *S. spontaneum*, all from the germplasm collection. All nine genome sequences appeared to represent a new species of *Mastrevirus* after their pairwise comparison using the species

demarcation tool (SDTversion1.2). Sequence similarity among sequences also suggested the presence of two strains of this new virus. This new species was found in plants that were introduced to Florida more than six decades ago.

#### **Mycosphaerellaceae associated with greasy spot of citrus in different climatic regions**

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Citrus greasy spot is distributed in humid areas in the Caribbean and Central America. The disease is characterized by yellow-brown spots on leaves and premature defoliation. Symptoms similar to greasy spot were observed in semi-arid areas in the Mediterranean Basin, although with low severity. The fungus *Zasmidium citri-griseum* (= *Mycosphaerella citri*) has been identified as the causal agent of greasy spot in the Caribbean and Central America, but disease etiology in the Mediterranean is unknown. A selection of 42 isolates of *Mycosphaerellaceae* obtained from greasy spot symptoms in humid areas in Ghana and Panama and arid regions in Spain and Morocco was studied. Isolates were characterized based on morphology, growth rate at different temperatures, multi-locus analysis (ITS, EF-1 $\alpha$  and RPB2), and pathogenicity tests. Isolates from Panama and Ghana (n=24) were identified as *Z. citri-griseum* whereas isolates from Spain and Morocco (n=20) were identified as *Amicosphaerella africana*. The optimum growth temperature of *Z. citri-griseum* was 27.9°C while *A. africana* grew better at 22.8°C. Isolates of *Z. citri-griseum* from Panamá were pathogenic to 'Valencia' sweet orange plants. None of the *A. africana* isolates from Spain induced symptoms in 'Ortanique' plants. These results confirmed *Z. citri-griseum* as the causal agent of greasy spot in Panama, being associated also with the disease in Ghana. Greasy spot symptoms in Spain and Morocco were associated with *A. africana*.

#### **First report of *Botrytis pseudocinerea* in blueberry fields in southern Chile**

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*Botrytis cinerea* is the most important pathogen in blueberry crops (*Vaccinium* spp.) in southern Chile, where the rainy and windy weather are highly favorable to its reproduction. Due we had observed a high morphological variability on *Botrytis* isolates from different commercial fields, the aim of this work was to perform a molecular study of isolates of *Botrytis* associated with blueberry gray mold. To estimate the diversity of this pathogen, 39 monospore cultures of *Botrytis* obtained from mature blueberry fruits from eight locations (Lat. 39°06'S to 41°28'S) and eight cultivars were genetically characterized. Their genotype was determined by PCR detection of transposons Boty and Flipper. In addition, coding fragment for the MS457 gene was sequenced and a phylogenetic tree was built using the Neighbor-joining and UPGMA clustering method. The genetic studies showed the presence of four genotypes, predominating Vacuma (57%) in the geographic region, then Boty (18%), Flipper (15%) and finally Transposa (10%). MS457 gene only was present in 10/39 isolates, its homology and phylogenetic analysis showed the presence of two sympatric populations of *Botrytis* on blueberry, allowed us to identify eight isolates as *B. cinerea* and two isolates as *Botrytis pseudocinerea*. In our knowledge this is the first report de *B. pseudocinerea* in blueberries in Chile.

#### **Identifying and characterizing fungal pathogens causing seedling diseases of soybean through a multi-state survey**

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Seedling diseases are common and very detrimental to soybean, *Glycine max*. They are caused by a variety of plant pathogens including fungi, bacteria, and oomycetes. The central goal of this project was to identify fungal pathogens causing seedling diseases of soybean in various soybean producing states. Two approaches were used for genus and species determination, including morphological features using microscopy and the sequencing of specific genetic loci. For sequencing, up to three different barcodes were targeted, the internal transcribed spacer (ITS), the elongation factor EF-1 $\alpha$ , and the intergenic spacer (IGS). The data produced from this project included the identification of approximately 150 fungal isolates per state from eight different states: Arkansas, Indiana, Illinois, Iowa, Nebraska, Michigan, Minnesota, and Kansas. Isolates were collected in 2012 and 2013 to help determine the predominant causal agents of seedling diseases of soybean in those surveyed locations. A variety of genera were isolated from the fields surveyed, with *Fusarium* species constituting approximately 74% of the isolates. Data analyses also involved determining associations and correlations between different factors, such as cultural practices, soil physical and chemical properties, irrigation practices, cropping history, and the frequency of the incidence of the species in the surveyed locations.

#### ***Fusarium* canker on holly in Norway**

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Holly (*Ilex aquifolium*) thrives well in the mild and wet coastal climate of southwestern Norway, where it is commonly harvested for Christmas decorations. During the last decade, a *Fusarium* sp. has frequently been found to damage both natural and cultivated stands, causing leaf drop, dead twigs, canker wounds and berry rot. The most severe outbreaks of the disease ever occurring were observed in late autumn 2015. Isolates of the fungus are most similar to *F. acuminatum* (97% identical TEF sequences). On one occasion, the fungus was detected on dead shoots of Nordmann fir (*Abies nordmanniana*). Inoculation tests proved it pathogenic to both host plants. Many holly stands are no longer intensively cultivated, and they have become very dense and overgrown by other vegetation, thus creating an ideal microclimate for fungal pathogens. In an investigation in a natural stand in 2006-2009, the quality of the holly improved by letting more air and light into the dense plantation by different combinations of pruning, thinning, clearance of unwanted trees and bushes, and grazing with sheep. Thus, it is possible to gain control with the disease by sanitation and proper management, and thereby increase both the quality and quantity for marketing. There is a potential market in other Nordic countries where the climate is too harsh for holly, but earlier attempts of export fell through due to quality issues.

#### **Fungal Diseases of *Stevia rebaudiana* Grown in Eastern Tennessee**

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Extract of *Stevia rebaudiana*, a plant native to Brazil and Paraguay, is a substitute for sugar to sweeten food and drink products. In the last five years, acreage of stevia for commercial production has increased significantly in the southeastern USA. Crops produced in large acreage monoculture are more susceptible to pathogens; potentially destroying whole crops or limiting crop yields. The aim of this study was to identify fungi recovered from diseased stevia field plants grown in Eastern Tennessee. Fungi were screened for pathogenicity in detached stevia leaf assays. Isolates that caused significant necrosis on detached leaves were identified and evaluated in replicated, greenhouse plant disease assays. Pathogens with significant potential to cause disease were identified as *Fusarium armeniacum*, *Botrytis cinerea*, and *Alternaria alternata*. Fungal identification was based on morphological characteristics and sequence identity of the internal transcribed spacer region of 18S ribosomal DNA, and translation elongation factor 1-alpha. Nucleotide amplicons obtained with PCR, were compared with published sequences in Fusarium-ID and the National Center for Biotechnology Information. In greenhouse assays, inoculated plants exhibited necrotic leaf lesions, similar to those observed in field plants. The three fungi were re-isolated from inoculated plants and identity was re-confirmed. Early identification and management of known stevia pathogens will help reduce crop loss.

### **Interaction between a rust fungus and *Colletotrichum truncatum***

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Mixed natural infection by *Uromyces* and *Colletotrichum* species in *Euphorbia hirta* lead to characteristic symptoms with rust pustules surrounded by black setose acervuli, formed exclusively in the presence of the rust fungus. Besides morphologically identified with precision, *U. euphorbiae*, was now characterized comparing sequences of the 28S rDNA with GenBank accesses of *Uromyces* species. Bayesian Inference (BI) and Maximum Likelihood (ML) analyses showed that *U. euphorbiae* is phylogenetically close to the type species, *U. appendiculatus*, and well distinguished from the remaining species considered, including those pathogenic to Euphorbiaceae. The *U. euphorbiae* type species reported on *E. hypericifolia* was collected in Albany, New York, USA in 1873. As the sequences from our specimen from Brazil, far from the type location, are not suited for its epitypification, it is here designated as a reference specimen, obtained from genomic DNA extracted from teliospores, and deposited in GenBank under number: KU133293, corresponding to our herbarium voucher: UB Mycol. Col. 22311. Two *Colletotrichum* isolates, one endophytic from symptomless areas of the leaves, and another from an acervulus close to rust pustules were isolated. For both isolates, sequences of the ITS, GAPDH, BTUB, and HIS were obtained and subjected to BI and ML analyses. The result clearly indicated, confirming the morphological analyses, that both isolates belong to the same species, *C. truncatum*.

### **Thielaviopsis punctulata Causes Black Scorch Disease on Date Palm in the United Arab Emirates**

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Date palm (*Phoenix dactylifera* L.) is one of the most important plants grown in the arid and semi-arid land that tolerates extreme environmental desert conditions. The main associated pathogens causing palm diseases are attributed to fungi and phytoplasma. In the United Arab Emirates (UAE), the causal agent of black scorch on date palm was found to be the fungal pathogen, *Thielaviopsis punctulata*. The pathogen was isolated from all tissues of diseased trees with virulent *Thielaviopsis punctulata*. Depending on the severity of the infection, symptoms included tissue necrosis, wilting, neck bending, death of terminal buds, and eventually plant mortality. This fungus, which was consistently isolated on potato dextrose agar from infected tissues, produced two types of conidia: the thick-walled aleuroconidia (chlamydoconidia) and phialoconidia (endoconidia). In addition, all target regions of 5.8S rDNA (ITS), 28S rDNA,  $\beta$ -tubulin and transcription elongation factor 1- $\alpha$  (*TEF1- $\alpha$* ) genes were amplified using polymerase chain reaction. We also found that the fungicide Score<sup>®</sup> inhibited the mycelial growth of *Thielaviopsis punctulata* both *in vitro* and *in vivo*. Altogether, the morphology of the fruiting structures, pathogenicity tests and molecular identification confirmed that the causal agent of symptomatic tissues is *Thielaviopsis punctulata*. This is the first report of the black scorch disease and the fungus *Thielaviopsis punctulata* on date palm in the UAE.

### **Fungal Species Associated with Olive Dieback of Branches and Evaluation of Cultivar Resistance to Botryosphaeriaceae species**

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Over two consecutive seasons, 16 olive orchards with trees exhibiting dieback symptoms of branches were surveyed in Southern Spain. The six most frequent fungal species recovered were morphologically, molecularly (by sequencing of the ITS, TUB, and LSU regions), and pathogenically characterized. The resistance of the 11 most important table cultivars to *Neofusicoccum mediterraneum* and *Botryosphaeria dothidea*, the causal agent of “escudete” (small shield) of fruit, was also studied by inoculation of branches and immature fruits, respectively. The species *Cytospora pruinosa*, *N. mediterraneum*, *Nothophoma quercina*, *Phoma incompta*, and *Phomopsis* sp. were identified. Only *N. mediterraneum* and *P. incompta* were able to induce the typical dieback symptoms and cankers that affected plants development. *N. mediterraneum* was the most virulent of the evaluated species although difference of virulence among their isolates were observed. The remaining fungal species were weakly pathogenic on potted plants. According to resistance tests, cv. Gordal Sevillana followed by the cvs. Manzanilla Cacerña and Manzanilla de Sevilla were the most susceptible to branch dieback caused by *N. mediterraneum*. Furthermore, the fruits of cv. Alorea de Atarfe followed by those of cvs. Ocal and Manzanilla de Sevilla were the most susceptible to *B. dothidea*. Knowledge of the etiology and cultivar resistance of these diseases will help to establish better control measures.

### **Identification of Alternaria species associated with tomato and potato early blight disease in Oman**

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In Oman, approximately 1400 hectares of tomatoes are planted each year with an annual production that exceeds 74000 tons. Tomatoes are ranked as the most important vegetable crop grown by local farmers. Early blight is a devastating disease in Oman and affects both tomato and potato production. In order to manage the disease more effectively, the Ministry of Agriculture and Fisheries in Oman initiated a project to characterize the pathogen population. Samples were collected from infected tomato and potato plants in commercial production fields during the 2014 and 2015 growing seasons from states in the north and south Al-Batinah governates in Oman. *Alternaria* spp. were isolated from symptomatic tissues. Microscopic examination of cultures from single spore isolations revealed three distinct species: *Alternaria tenuissima*, *A. alternata* and *A. solani*. Molecular identification was achieved through amplification and sequencing of internal transcribed spacer (ITS) and histone 3 gene regions. Phylogenetic analysis of the ITS gene clearly separated *Alternaria solani* from the other two species. Partial coding of histone 3 gene revealed three clades representing *A. alternata* (440bp), *A. solani* (489bp) and *A. tenuissima* (546bp). Pathogenicity tests of the sample isolates on detached tomato leaves of cultivar ‘Ginan’ showed brown lesions similar to symptoms observed in field. No symptoms were observed in water-inoculated control tomato leaves.

### **Morphological and Molecular Characterization of Plant-Parasitic Nematodes Populations from Corn Fields in Ohio**

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Results from an Ohio survey in 2013 and 2014 showed that root-lesion nematode, among the most economically important plant-parasitic nematodes, was detected in more than 80% of the 425 fields sampled. Other plant-parasitic nematodes extracted were the lance, stunt, dagger, pin and spiral nematodes, found in 48, 37, 57, and 94% of fields, respectively; however, the impact of these nematodes on corn in Ohio remains unclear. Because damage caused by nematodes varies among genera and species within genera, it is necessary to characterize these nematodes to species. Nematodes were killed, fixed, and mounted before examination with a compound microscope, and identified to species based on quantitative and qualitative morphological characteristics with the aid of diagnostic keys. Three species with distinct morphological features of the genus *Pratylenchus* were identified as *P. penetrans*, *P. neglectus*, and *P. thornei*. Two species of *Tylenchorhynchus* were identified as *T. silvaticus* and *T. nudus*. Species of the lance, pin, spiral and dagger nematodes, namely *Hoplolaimus galeatus*, *Paratylenchus neoamblycephalus* and *Helicotylenchus pseudorobustus* were also identified based on morphology. Species identity will be further confirmed based on analyses of the D2-D3 regions of 28S rDNA gene and ITS rDNA gene. Findings from this study will be useful for establishing experiments to develop damage relationships and management programs for corn nematodes in Ohio.



### **Vermiform plant-parasitic nematodes on soybean in North Dakota and their relationship with soybean cyst nematode**

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Plant-parasitic nematodes (PPN) are an important group of pests on soybean. The occurrence and population densities of PPN other than soybean cyst nematode (SCN; *Heterodera glycines*) are unknown in soybean fields of North Dakota. A survey was conducted in 2015 in soybean fields or fields with history of SCN to ascertain the incidence and abundance of vermiform PPN and their possible association with SCN. Vermiform and cyst nematodes were extracted separately from each sample (100 cm<sup>3</sup> soil) and then identified and quantified. Out of the 155 soil samples collected from 14 counties in North Dakota, 144 were found to be infested with PPN. Eight genera of vermiform PPN were detected including *Helicotylenchus* (incidence: 49%; highest density: 1,800; average density: 174), *Tylenchorhynchus* (41%; 340; 30), *Paratylenchus* (37%; 2,480; 151), *Pratylenchus* (19%; 245; 9), *Xiphinema* (7%; 180; 4), *Paratrichodoros* (4%; 60; 1), *Hoplolaimus* (3%; 140; 2), and *Mesocriconema* (1%; 300; 2). Both SCN juveniles (24%; 1,200; 46) from soil and SCN eggs (56%; 21,540; 501) from cysts were counted, but weak correlation was found between them for all samples ( $r = 0.489$ ;  $p < 0.0001$ ). Poor or no correlations between population densities of each genus of vermiform nematodes and SCN were observed with  $r$  ranging from -0.307 to 0.248. More soil samples will be collected in 2016 to confirm the occurrence and abundance of nematodes in soybean fields and the relationship of vermiform nematodes with SCN.

### **Occurrence of *Phytophthora syringae* causing Phytophthora fruit rot in apple in Chile**

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Chilean apple industry is one of the major exporter of fresh apples worldwide with an export volume of 628,247 ton. Maule Region (35°25') is the most important apple production region in Chile with a 60% of production of fresh apple. The fungal diseases in postharvest, is one of the major problems for Chilean industry of fresh apple. Recently was described speck rot caused by *Phacidiopycnis washingtonensis* in apple in Chile. During survey for postharvest diseases of apple in 2014-2015, a postharvest rot was observed on stored apple cv. Jonagold and Pink Lady collected in packinghouses. Symptomatic apples (n=40) were characterized by an initial light brown skin discoloration with spongy to firm flesh texture. With the objective to isolate the causal agent, small fragments were collected from diseased tissues and aseptically placed on Corn Meal Agar and PDA. A *Phytophthora* sp. was consistently isolated. Eight representative *Phytophthora* isolates were characterized for morphological and molecular identification, pathogenicity tests and sensitivity to fungicide. Based on morphology and phylogenetic analyses, this fungus was identified as *Phytophthora syringae*. All isolates grew at 0°C. Inoculated apple fruits developed necrotic lesions. The isolates were sensitive to dimethomorph. To our knowledge, this study is the first description of *P. syringae* causing Phytophthora fruit rot on apple during postharvest in Chile.

### **Phytophthora root and crown rot on field-grown lavender plants in 2015**

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*Phytophthora nicotianae* has a very wide host range, including numerous ornamental plants. In Jun 2015, *P. nicotianae* was isolated from lavender plants and rooted cuttings (*Lavandula angustifolia* and *L. ×intermedia*) with symptoms of Phytophthora root and crown rot (PRCR). The plants recently had been planted in a field in northwestern SC. When this was brought to the attention of the US Lavender Growers Association (USLGA), it was discovered that other growers suspected problems with PRCR—particularly on recently-planted, nursery-grown plants. USLGA members were encouraged to send samples to Clemson University to test for *Phytophthora* spp. Between Jul and Nov 2015, plant and soil samples were received from 12 states; all plants were cultivars of *L. angustifolia* or *L. ×intermedia*. Samples from 11 states were diagnosed with PRCR; *Phytophthora* spp. were recovered from roots and crowns by isolation on selective media and from soil with a baiting bioassay. Isolates were tentatively identified by morphology, but species identification will be confirmed by DNA sequences. Three species of *Phytophthora* were isolated: *P. nicotianae* was isolated most frequently—from samples from 11 states; *P. palmivora* was found in samples from 3 states; and *Phytophthora* sp. was found in one sample. Previously, PRCR has been reported on English lavender, *L. angustifolia*, but not on hybrid lavender, *L. ×intermedia*. Lavender plants infected with *Phytophthora* spp. may have come from the nursery. Authors acknowledge CONICYT PhD fellowship (MV), CONICYT-GO (MV), USM 131562, FONDECYT 1151174& CBDAL grants(MS).

### **Four species of *Phytophthora* recovered from roots of American and hybrid chestnut seedlings and associated soils in the southeastern US**

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Since Phytophthora root rot (PRR) was first described on American chestnut in 1932, the only species of *Phytophthora* reported as a causal agent has been *P. cinnamomi*. In Europe, however, multiple species of *Phytophthora* are involved in PRR of native chestnut trees. In 2009, the USDA Forest Service began evaluating American, Chinese, and hybrid chestnut seedlings in forest test plots in NC, TN, and VA, but PRR caused some seedlings to die prematurely. The objective of this research was to determine if multiple species of *Phytophthora* may be causing PRR of American and hybrid chestnut seedlings. A total of 260 root and 454 soil samples were assayed from 2010 to 2014. *Phytophthora* spp. were recovered by isolation from roots on PARPH-V8 selective medium and baiting soil with rhododendron and camellia leaf pieces. All isolates were tentatively identified based on morphology; species identifications will be confirmed by DNA sequences. *P. cinnamomi* was recovered most frequently—from 41 (16%) root and 109 (24%) soil samples. However, *P. cambivora* and *P. heveae* were also recovered—from 18 (7%) and 2 (<1%) root samples and 18 (4%) and 17 (4%) soil samples, respectively. At a separate test site in SC, *P. drechsleri* was isolated from roots of several hybrid chestnut seedlings. This is the first report of *Phytophthora* species other than *P. cinnamomi* associated with PRR of pure and hybrid American chestnut. These species are being tested for pathogenicity to American chestnut.

### **Two new races and several novel strains of the spinach downy mildew pathogen *Peronospora farinosa* f. sp. *spinaciae***

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Downy mildew disease, caused by *Peronospora farinosa* f. sp. *spinaciae* (Pfs), is the most economically important disease of spinach. Current high-density spinach production provides conducive conditions for disease development, particularly for the increasing acreages of organic spinach. The use of resistant cultivars to manage downy mildew exerts strong selection pressure on the pathogen and many new races of Pfs have emerged in recent years threatening spinach production worldwide. To monitor race diversity of Pfs, downy mildew samples were collected from major spinach production areas and tested for race identification with a standard set of differentials. Two new races (races 15 and 16, sanctioned by the IWGP) and several novel strains have been identified in the past 3 years. The disease reactions of Pfs15 (isolate UA4712) was identical to that of race 4 except the race 4 could not overcome the RPF9 locus resistance and race 15 could. The RPF1, 2, 4, and 6 loci provided resistance to Pfs15. Pfs16 (isolate UA201519B) could overcome the resistance of RPF2, 4, 5, 9, and 10 but not RPF1, 3, and 6. One novel strain (UA1014APLP) could overcome all known spinach resistant loci, but it only infects cotyledons and not true leaves of some cultivars. None of the 360 USDA spinach germplasm accessions tested has resistance to Pfs16 or UA1014APLP. Improved resistance is needed for protection of spinach to the highly variable spinach downy mildew pathogen.

### First report of tomato late blight disease in Oman

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Prior to 2013, tomato late blight had not been reported from the Sultanate of Oman. Tomato is an important vegetable crop, with approximately 1400 ha of tomatoes in Oman. However, in 2013 a disease resembling late blight was observed in several states, with some farms reporting destruction of 100% of the area planted with tomatoes and decrease in production of 30-90% for other farms. The causal agent of late blight, *Phytophthora infestans*, was successfully isolated from infected plant tissues in February and March 2013. Identification of *P. infestans* was achieved through microscopic observation and molecular analyses. Sporangia were lemon shaped, semipapillate and caduceus ranging from 36 to 41 µm in length and 13 to 30 µm in width. Internal transcribed spacer (ITS) and cytochrome c oxidase 1 (Cox1) gene regions were amplified and sequenced. BLAST search for ITS and Cox1 sequences of isolates collected from Oman showed 100% similarity with sequences of *P. infestans* isolates available in genbank (NCBI). To confirm pathogenicity of *P. infestans* in tomato, three isolates were selected to fulfill Koch's postulates. A spore suspension with concentration of  $2.5 \times 10^4$  sporangia/ml of each isolate was inoculated on tomato leaves of the cultivar "Ginan" and incubated at 18 Co for 12 days. Symptoms of water soaked and greyish black lesions were observed on inoculated leaves while tomato leaves inoculated with sterile water remained healthy.

### Red clover vein mosaic virus (RCVMV), a recently reported disease agent from alfalfa, cultivated crops and weeds in Saudi Arabia

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During a two years survey (2012-2013), 1166 samples were collected from symptomatic alfalfa plants in the major alfalfa-producing regions in Saudi Arabia to detect RCVMV and other viruses. Fourteen samples were positive by DAS-ELISA to RCVMV. In 2014, a detailed study was performed to determine the status and to characterize RCVMV where 315 samples were collected from the three utmost producing regions. Fifty six samples were positive, which were confirmed by RT-PCR. Re-PCR products of 932 bp were obtained from the cDNA of RCVMV-infected plants. In the host range study five (alfalfa, black eye pea, cowpea, Gomphrena and glutinosa tobacco) out of 17 plant species resulted in variable symptoms which were confirmed by ELISA, PCR and nucleic acid hybridization assay. This study reports for the first time that cowpea, black eye pea and *Nicotiana glutinosa* are hosts for RCVMV. This is the first detailed report regarding RCVMV infecting alfalfa in Saudi Arabia and its characterization. Data regarding infection of field crops and weeds with this virus will be included. Sequence Homology tree revealed high CP sequence similarity between the Saudi isolates of RCVMV (99-100%), but low CP sequence similarity between the Saudi isolates and other foreign isolates of RCVMV (35%). Phylogenetic analysis of sequences of the Saudi isolates of RCVMV and 40 other CP sequences of Carlavirus species from GenBank grouped in the same clade though they had low homology percentages.

### Rubus yellow net virus: An integrated element in the raspberry genome

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*Rubus yellow net virus* (RYNV) infects *Rubus* spp. causing a serious decline when present in mixed infections and a vein chlorosis in indicator plants. RYNV belongs to the family *Caulimoviridae*, which can exist as infectious particles (exogenous) or integrated elements (endogenous). It was observed that RYNV PCR-positive plants often did not induce symptoms in bioassays, suggesting an integrated element. Molecular techniques were used to discriminate between exogenous and endogenous RYNV sequences. Reverse transcription-PCR using RYNV specific primers after DNase treatment produced positive results for the virus in graft transmissible isolates only. To confirm these results, rolling circle amplification on DNA preparations from these plants resulted in amplicons whose sequence belonged to RYNV. This suggested that graft-transmissible isolates contained an exogenous version of the virus. In Southern blot analysis using *Rubus* genomic DNA digested with *Bam*HI (one recognition site in the RYNV genome), samples that were positive in bioassays for RYNV gave a single band the size of the RYNV genome, while plants with non-transmissible isolates produced three extra bands. Next generation sequencing and appropriate bioinformatics programs are being used to identify the specific integration sites. These results show that RYNV can be integrated into the raspberry genome and highlight the need to consider this phenomenon in future *Rubus* quarantine and certification programs.

### Raspberry leaf curl disease

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Raspberry leaf curl disease (RLCD) was first reported in the late 19<sup>th</sup> century in eastern Canada and northeastern U.S. and is a sporadic destructive disease of red raspberry. Symptoms include leaf curling, leaf distortion, leaf chlorosis, shoot dwarfing, and shoot proliferation. Infected plants produce crumbly, unmarketable fruit, show severe decline within a few years and are much more susceptible to winter injury, which can kill infected plants. RLCD is vectored by the aphid *Aphis rubicola* in a persistent manner. Based on transmission properties it is suspected RLCD is caused by a virus or virus complex. To determine the viral etiology of RLCD, total nucleic acids were extracted from two red raspberry plants showing typical symptoms of RLCD, digested with DNase, rRNA removed and finally subjected to next generation sequencing on the Illumina Miseq platform using the paired end 150 bp kit. Sequences were analyzed with the VirFind pipeline and specific primers were designed to validate potential virus-like sequences. RLCD infected plants were grafted to blackberries and raspberries, and vector studies with aphids were undertaken. The virome of the plants exhibiting RLCD-like symptoms will be presented.

### Current Status of Grapevine Virus Diseases in British Columbia

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Grapevine leafroll disease (GLRD), caused by Grapevine leafroll associated viruses (GLRaVs), is considered the most wide spread virus disease of grapevine (*Vitis vinifera* L.) worldwide. British Columbia (BC) is the second largest grape-growing region in Canada with most of the acreage planted in the Okanagan Valley. To understand the epidemiology of GLRaVs in BC vineyards, a comprehensive survey was conducted during the 2014 and 2015 growing seasons throughout all wine growing regions of BC. The status of GLRaVs in BC was determined by analyzing a total of 2740 random composite samples representing 12 white and 9 red cultivars. Both ELISA and PCR based diagnostic test results showed GLRaV-3 to be the most prevalent (23.9%) followed by GLRaV-2 (7.1%), GLRaV-1 (2.7%) and GLRaV-4 (2.7%). GLRaVs mixed infections were detected in different combinations from composite samples with GLRaV-1 + GLRaV-3 (2.5%) to be the most common, followed by GLRaV-2 + GLRaV-3 (2.1%) and GLRaV-3 + GLRaV-4 (0.9%). Our results also revealed the presence of *Grapevine fan leaf virus*, *Grapevine fleck virus*, *Grapevine Pinot gris virus*, *Grapevine red blotch associated virus* and *Rupestris stem pitting associated virus* (RSPaV). These results along with information generated on the disease progress and insect vectors in BC will emphasize the importance of a clean plan network program for sustainability of Canadian wine grapes.

### The prevalence of grapevine leafroll and red blotch diseases in Washington vineyards

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Grapevine leafroll disease (GLD) is a significant constraint to the production of wine grapes (*Vitis vinifera*) in Washington State. *Grapevine leafroll associated virus* (GLRaV) 1, 2, 3, and 4 have so far been documented, with GLRaV-3 being the most widely distributed in Washington vineyards. However, recent reports of the occurrence of grapevine red blotch disease (GRBD) have raised concerns about the extent of its distribution in Washington vineyards. Since the symptomatology of GLD and GRBD is highly similar in wine grape cultivars, reliable information on the prevalence of these two diseases is necessary for deploying appropriate disease mitigation strategies. Towards this objective, surveys of vineyards were conducted during 2014 and 2015 seasons in seven American Viticultural Areas of Washington. A total of 1,469 samples collected from fourteen red-berried and five white-berried wine grape cultivars were tested for the presence of GLRaV-3 and *Grapevine red blotch-associated virus* (GRBaV) by polymerase chain reaction (PCR)-based diagnostic assays. The PCR results were further confirmed by cloning and sequencing of virus-specific amplicons. The combined data showed that 83.7% and 13.7% of samples were positive for GLRaV-3 and GRBaV, respectively, indicating that GLD is predominant relative to GRBD in Washington vineyards. A multiplex PCR assay has been optimized for high throughput testing of grapevine samples for the presence of GLRaV-3 and GRBaV.

#### **Association of *Grapevine rupestris stem pitting-associated virus* and *Grapevine Pinot gris virus* in declining grapevines on Schwarzman rootstock**

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In the fall of 2014, in Napa County, California, four-year-old grapevines of Cabernet Sauvignon (*Vitis vinifera*) on Schwarzmann (*V. riparia* X *V. rupestris*) rootstock were found to exhibit red canopy and vine decline. Upon examination of the woody cylinder, the rootstocks, but not the scions, had extensive stem pitting. To investigate the causal agent(s), nucleic acid extracts from affected grapevines were analyzed for viruses by RT-PCR assays. These vines tested positive for the Syrah strain of *Grapevine rupestris stem pitting-associated virus* (GRSPaV-Syrah) and negative for five leafroll-associated viruses, three vitiviruses, and Grapevine red blotch-associated virus. Further examination by deep sequencing of a cDNA library, prepared from the dsRNA fraction of cambial scrapings from the affected trunks, and subsequent bioinformatics analysis, revealed the presence of *Grapevine Pinot gris virus* (GPGV) in addition to GRSPaV-Syrah. Canopy surveys conducted in the fall of 2015 indicated that >40% (n=342) of the vines had declined. It is likely that the association of GPGV and GRSPaV may have a synergistic effect resulting in the stem pitting symptom.

#### **Survey of *Arabis Mosaic* and *Grape Leaf Roll Associated virus* in Maryland vineyards**

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*Arabis mosaic virus* (ArMV) and *grape leaf roll associated virus* (GRLaV) cause significant crop loss in grapes. However, their natural incidence in Maryland vineyards is not known, and several growers have reported symptoms similar to those caused by these viruses. To find their distribution, 15 vineyards were surveyed during August to September 2015 in Maryland. Up to six varieties per vineyard were examined for virus symptoms. Up to five samples (symptomatic, if present) were collected from each variety. Two hundred seventy samples were tested for each virus group, including the three most symptomatic samples per variety. These samples were tested using a double-antibody sandwich (DAS)-enzyme-linked immunosorbent assay (ELISA) kit developed by Bioreba for GRLaV, and by Agdia for ArMV. Three groups of GRLaV (GRLaV-1+3 and GRLaV-2) were tested in two groups. Forty percent of vine samples collected were symptomatic for GRLaV and 29% were symptomatic for ArMV. Only ten percent of samples were found positive for ArMV. Similarly, only 1.1% of samples tested were positive for (GRLaV 1 + GRLaV 3) and 1.5% of samples were positive for GRLaV-2. This result indicated that many plants exhibiting symptoms similar to GRLaV and ArMV were negative for these viruses. This may be because symptoms observed in the field were produced by causes other than the virus tested. Further testing of such vines for other virus and virus-like pathogens may help growers to manage this problem.

#### **First report of *Watermelon chlorotic stunt virus* in rain-fed farms in Usfan, Saudi Arabia**

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Watermelon (*Citrullus vulgaris* L.), an important crop in Saudi Arabia, is cultivated in the lowlands and wadis following seasonal winter rainfall, along the western coast. Production of watermelon faces constraints imposed by abiotic stresses and biotic diseases, and insects, including whiteflies. During spring 2015, >95% of watermelon plants growing on a farm near Usfan exhibited foliar chlorosis and mottling, and overall stunting symptoms, reminiscent of those caused by *Watermelon chlorotic stunt virus* (WmCSV) (*Begomovirus*, *Geminiviridae*). Total nucleic acids were isolated from leaves of three symptomatic watermelon plants, and subjected to rolling circle amplification (RCA). Libraries prepared for the RCA amplicons were subjected to Illumina MiSeq, and reads were assembled using SeqMan NGen software. BLASTn analysis of assembled contigs revealed the presence of the DNA-A and DNA-B genomic components most closely related to the Old World bipartite WmCSV. The three DNA-A components, at 2752 bp, shared 99-100% nucleotide (nt) identity with each other, and 99% nt with a WmCSV isolate from Leith, Saudi Arabia [KJ939448]. The three DNA-B component sequences, at 2757 bp, shared 96-97% nt identity with each other, and 96% nt identity with the DNA-B component of an isolate, also from Leith [KJ939447]. This is the first report of WmCSV in the Leith, a farming community separated from Usfan by a vast, inhospitable desert.

#### **Necrotic Streaking of Asiatic Lilies caused by *Plantago asiatica mosaic virus* in Chile**

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During the spring of 2015 a survey was done in Quillota, in greenhouses with Asiatic hybrid lilies for cut flower. Symptomatic plants were found in four different cultivars. Symptoms were brown necrotic streaking associated with irregular leaf chlorosis, and severely infected plants also showed brown streaks in the stem and irregular necrosis in tepal tips. Only in cv. Litouwen it was observed that microbulbs replaced flower buds. Tissue extracts from symptomatic plants were obtained and RT-PCR of gene RdRp was performed employing specific primers for Potexvirus. The obtained sequences revealed 99% identity with the consensus sequences of PIAMV. After positive results for PIAMV, a pathogenicity test was performed on plants of two cultivars of Asiatic lilies. Two isolates of PIAMV were inoculated in three plants for each isolate, and other three plants were left as controls. Forty-five days after inoculation, plants from Asiatic cv. Indian Summerset showed a shorter stem, necrotic streaks in leaves and in the stems and chlorosis in irregular pattern. Litouwen plants showed symptoms of decreased growth, brown necrotic streaks in the leaves associated with chlorosis and microbulbs instead of flower buds. All symptomatic plants tested positive with specific primer for PIAMV and control plants tested negative. Koch's postulates were fulfilled for PIAMV on *Lilium* plants and the disease was called Necrotic Streaking of Lily caused by PIAMV affecting Asiatic cultivars.

#### ***Trichosanthes latent virus* (TrLV), a novel potyvirus from *Trichosanthes cucumeroides* in Japan**

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Only a few studies have been conducted on incidence of plant viruses on wild cucurbit weeds in Japan. As wild and horticultural cucurbits often share same viruses, epidemiological survey as well as precise identification of causal virus(es) is required for the efficient control strategy. We once reported un-common potyvirus tentatively named as JK virus isolated from *Trichosanthes cucumeroides* (Kim *et al.*, 2011). By additional surveys, most of

collected *T. cucumeroides* in Kanto region were found to be infected with JK virus showing high co-incidence with *Papaya ringspot virus* (PRSV). On the contrary, as far as examined, JK virus was not detected from any wild cucurbit samples including *Trichosanthes* spp. in Okinawa Prefecture where *Papaya leaf distortion mosaic virus* (PLDMV) infection on cucurbit weeds have been reported (Maoka and Hataya, 2005). Mechanical inoculation of JK virus indicated a narrow host range limited to family *Cucurbitaceae* and showed symptomless or mild symptom when back-inoculated to *T. cucumeroides*. Compared with sequences of CI or CP regions from other potyviruses, JK virus shared less than 80% identity with PLDMV which was the most closely related potyvirus. Therefore, JK virus can be considered as a novel potyvirus species, for which the name as *Trichosanthes latent virus* (TrLV) is proposed.

#### **Identification and characterization of Zucchini tigre mosaic virus on creeping cucumber in Japan**

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Creeping cucumber (*Melothria pendula*), a wild cucurbit weed, showing mottle and distortion symptoms was observed in Okinawa main island, Japan in September 2015. Total RNA using TRIzol reagent extracted from collected samples was used for reverse transcription-polymerase chain reaction (RT-PCR) with degenerate primers (CIFor and CIRev) for potyviruses (Ha *et al.*, 2008) followed by cloning and sequencing. Obtained 637 nucleotides (nts) sequence of partial CI region from isolate Oki-47 showed 86% with corresponding sequence from Zucchini tigre mosaic virus (ZTMV; accession no. KC345554) as a highest identity. When the whole coat protein (CP) coding region was considered, the nt and amino acid (aa) sequence identities shared 94% and 97% with *Papaya ringspot virus* (PRSV; accession no. AB583208) respectively. The compared PRSV isolate was re-classified as one of ZTMV isolates by Romay *et al.* (2014). Serological test by western blotting assay revealed that isolate Oki-47 was closely related to PRSV, but not to *Papaya leaf distortion mosaic virus* (PLDMV). Absence of PRSV, PLDMV, and *Zucchini yellow mosaic virus* (ZYMV) were also confirmed which are known as major causal potyviruses infecting to cucurbits in Japan. This is the first report of ZTMV incidence in Japan as well as creeping cucumber for its natural host.

#### **Complete nucleotide sequences of a new bipartite begomovirus from *Malvastrum* sp. plants with bright yellow mosaic symptoms in South Texas**

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Two isolates of a new bipartite *Begomovirus*, tentatively named as *Malvastrum bright yellow mosaic virus* (MaBYMV), were molecularly characterized from naturally-infected *Malvastrum* sp. plants showing bright yellow mosaic disease symptoms in South Texas. The host plant genus identity was determined by DNA barcoding of the non-coding *trnH-psbA* intergenic spacer region and the *matK* plastid gene. Restriction enzyme-digested rolling cycle amplification products from symptomatic plants were used as template in PCR to amplify complete virus genome segments with pairs of newly-designed abutting primers. The obtained ~2.7 Kb fragments were cloned and sequenced. Six complete DNA-A and five DNA-B genome sequences of MaBYMV obtained from the two isolates ranged in length from 2,608-2,609 nucleotides (nt) and 2,578-2,605 nt, respectively. Both genome segments share a 178-180 nt common region. In pairwise comparisons, complete DNA-A and DNA-B of MaBYMV were most identical (87-88% and 79-81%, respectively) and phylogenetically related to corresponding sequences of *Sida mosaic Sinaloa virus*-[MX-Gua-06]. Further analysis showed that MaBYMV is a putative recombinant virus, thus supporting the notion that malvaceous hosts may be influencing the evolution of several begomoviruses. The design of new diagnostic primers enabled the detection of MaBYMV in cohorts of *Bemisia tabaci* collected from symptomatic *Malvastrum* sp. plants, thus implicating whiteflies as potential vector of the virus.

#### **Infectivity analysis of strain L of chickpea chlorotic dwarf virus, a cotton mastrevirus, in *Nicotiana benthamiana* plants**

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Cotton is damaged by a group of viruses, responsible for cotton leaf curl disease complex, belongs to the genus *Begomovirus*; family *Geminiviridae*. The present study unveils another virus which belongs to genus of the same family causing leaf curl disease in cotton. Agrobacterium-mediated inoculation, of strain L of the chickpea chlorotic dwarf virus (CpCDV) (mastrevirus) isolated from cotton plants, to *Nicotiana benthamiana*, alone or in combination with other begomovirus showed a remarkable difference in symptoms from very mild symptoms to severe cup shaped with stunted growth. The variability in symptoms and their development in other parts of tested plants and subject viruses, belonging to different genera, perhaps owing to difference in ORFs of subject viruses.

#### **Nucleotide sequence and genome organization of a novel *Panicovirus* from Bermuda grass**

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Bermuda grass (*Cynodon dactylon*) is widely used in tropical and subtropical areas for golf greens, athletic fields, and landscapes. Samples of Bermuda grass displaying symptoms of decline were tested at the University of Minnesota plant virology lab. Spherical virus particles (28-30 nm) were observed by transmission electron microscopy. Total RNA was extracted and sent for Next Generation Sequencing (NGS) on an Illumina platform. Contigs were assembled using CLC Workbench, and analyzed using BLAST searches against reference genomes. A contig of about 4 kb contained four predicted ORFs in an arrangement typical of viruses in the family *Tombusviridae*. Overlapping primer pairs were designed from the consensus sequence for RT-PCR verification and sequence confirmation. All amplicons were cloned and the resulting sequences matched the original NGS contig. Amino acid sequence comparisons of the putative replicase (RdRp) and movement and coat proteins (CP) showed sequence similarities in the range of 37-66% compared to *Thin paspalum asymptomatic virus*, *Cocksfoot mild mosaic virus*, and *Panicum mosaic virus*. Phylogenetic analyses using the CP and RdRP of this newly identified virus place it in the genus *Panicovirus*. The sequence of this Bermuda grass virus is sufficiently different from other viruses to conclude it is a new member of the genus *Panicovirus*, family *Tombusviridae*.

#### **Identification of virus species infecting pepper in Guangdong province of China**

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Viral disease is one of main diseases of pepper production in Guangdong province of China. During 2013-2015, we surveyed the occurrence of the viral disease and identified the viruses infecting pepper. The diseased samples of pepper were collected randomly from the main pepper-growing areas of Guangdong. The diseased samples of the same survey location were mixed and extracted total RNA for deep sequencing of small RNA. The viruses were verified by RT-PCR, respectively using the specific primers designed according to the assembling sequences. The diseased plants in fields exhibited diversiform symptoms, including stunting, mosaic, chlorosis, leaf narrowing and distortion, and mottled. The sequence analysis results showed that there were 16 species viruses infecting pepper, including *Chilli ringspot virus*, *Broad bean wilt virus*, *Cucumber mosaic virus*, *Pepper vein yellows virus*, *Chilli vein mottle virus*, *Pepper vein mottle virus*, *Pepper yellow leaf curl virus*, *Capsicum chlorosis virus*, *Tobacco mild green mosaic virus*, *Pepper cryptic virus*, *Potato virus Y*, *Pepper mild mottle virus*, *Watermelon silver mottle virus*, *Tobacco mosaic virus*, *Bell pepper endornavirus*, *Nicotiana tabacum pararetrovirus-like*. The co-infection with two, three and more than three viruses accounted for 6.02%, 21.29% and 69.88% in one

diseased sample, respectively. It is concluded that the number of virus species infecting pepper crop was abundant and co-infection was common in Guangdong of China.

#### **Beet curly top virus strains associated with sugar beet in Idaho, Oregon, and a survey collection**

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Curly top of sugar beet is a serious yield limiting disease in semi-arid production areas caused by *Beet curly top virus* (BCTV) and vectored by the beet leafhopper. The primary means of control for BCTV is host resistance, but effectiveness of resistance can vary with BCTV strain. BCTV variation was last investigated in Idaho and Oregon during a 2006-2007 survey, but changes in disease severity suggested a need for reevaluation. Therefore, 406 leaf samples symptomatic for curly top were collected from sugar beet plants in commercial sugar beet fields in Idaho and Oregon from 2012 to 2015. DNA was isolated and the BCTV strain composition was investigated based on polymerase chain reaction (PCR) assays with strain specific primers for Severe (Svr) and California/Logan (CA/Logan) strains and nonspecific primers that amplified Worland (Wor)-like strains. The 2006-2007 ID/OR BCTV positive samples from sugar beet included the following strains: 87% Svr, 7% CA/Logan, and 60% Wor-like. BCTV strain distribution in the new survey averaged 2% Svr, 30% CA/Logan, and 87% Wor-like. Whole genome sequencing (GenBank accessions KT276895 to KT276920) with overlapping primers, suggests that the Wor-like strains included Wor, Colorado (CO), and a previously undescribed strain designated Kimberly1 (Kim1). These data indicate there was a shift from Svr being one of the dominant BCTV strains in commercial sugar beet fields in 2006-2007 to being undetected at times in recent years.

#### **Virus diseases: A major threat to high value vegetable crops in Bangladesh and Nepal**

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The Feed the Future Innovation Lab for Integrated Pest Management (IPM IL), a USAID-funded program managed by Virginia Tech, has been working on identifying major threats to vegetable production in Bangladesh and Nepal. Surveys have been conducted in major production areas including those of smallholder farmers. Results show a trend of emerging and re-emerging virus disease in cucurbits including cucumber, squash, pumpkin and other gourds, tomato, pepper, potato, and okra. Research is focused on identifying sources of virus, with a special emphasis on insect vector and seed transmission pathways. The IPM IL is working with farmers, researchers, and local and international NGOs in Bangladesh and Nepal to address management of these virus diseases, and developing ecologically-based and environmentally friendly IPM strategies that reduce the reliance on synthetic pesticides and improve yield while at the same time reducing damage to the environment and improving nutrition and health. IPM packages have been developed and are being disseminated through farmer participatory approaches including IPM demonstration plots and farmer groups.

#### **Tomato yellow mottle virus (ToYMoV): Characterization of a newly emergent begomovirus associated with tomato disease in Costa Rica**

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Tomato production in Costa Rica is affected by diseases caused by whitefly-transmitted begomoviruses. Since the early 1990s, the main virus involved was the New World (NW) bipartite *Tomato yellow mottle virus* (ToYMoV), which was associated with stunting and yellow mottling. To fulfill Koch's postulates, putative full-length were obtained. *Nicotiana benthamiana* and tomato seedlings inoculated with the ToYMoV DNA-A and DNA-B clones developed stunting and yellow mottling of leaves, confirming the infectivity of the clones. Sequence analysis revealed that ToYMoV is closely related to bipartite tomato-infecting begomoviruses from Mexico and Guatemala. Multimeric clones were generated and used to assess the host range of ToYMoV. More recently, the NW bipartite *Tomato leaf curl Sinaloa virus* (ToLCSiV) and the invasive Old World (OW) monopartite *Tomato yellow leaf curl virus* (TYLCV) were introduced to Costa Rica where they have caused yield losses. Pseudorecombinants made between infectious DNA-A and DNA-B clones of ToYMoV and ToLCSiV did not induce symptoms. Tomato plants inoculated with cloned DNAs of TYLCV, ToLCSiV and ToYMoV alone or in mixtures revealed that symptoms of ToLCSiV and ToYMoV appeared earlier than those of TYLCV. However, within 14 days after inoculation, TYLCV symptoms became dominant, although ToLCSiV and ToYMoV were still detected at high levels. These results will be discussed in terms of synergy among these viruses.

#### **Validation of a novel gene target for detection and quantification of *Clavibacter michiganensis* subsp. *nebraskensis*, the causal agent of Goss's wilt**

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Goss's wilt of maize is a reemerging and significant disease caused by the bacterium *Clavibacter michiganensis* subsp. *nebraskensis* (*Cmn*). Despite its expansion in North America, molecular tools for diagnosing and studying the epidemiology of this disease remain limited. Here we report the identification of CMN\_01184 as a novel gene target and demonstrate its use for development of conventional PCR (cPCR) and quantitative PCR (qPCR) assays for detection and quantification of *Cmn*. Primers designed for cPCR and qPCR were evaluated for specific amplification of CMN\_01184 using a diverse collection of 129 bacterial and fungal isolates, including multiple corn pathogens, environmental organisms from agricultural fields, and all known subspecies of *C. michiganensis*. Of these, only isolates of *Cmn* tested positive for CMN\_01184. To evaluate the utility of CMN\_01184 for *in situ* detection of *Cmn*, cPCR and qPCR based on this target gene was performed on infested field soil and naturally infected corn leaves containing *Cmn*. CMN\_01184 was amplified only in soil samples and leaves containing *Cmn*. Finally, in greenhouse studies, systemic infection of corn leaves by *Cmn* was quantified over a two-week infection period using the qPCR assay based on CMN\_00184. The results demonstrate collectively that specific and sensitive PCR assays based on CMN\_01184 can aid in diagnosis as well as studies of the epidemiology and ecology of Goss's wilt.

#### **In-field detection of the select agent *Rathayibacter toxicus* using loop-mediated isothermal amplification**

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Early, accurate and sensitive detection of *Rathayibacter toxicus* by plant health officials would improve inspections of imported annual ryegrass hay and seed at ports of entry and enhance in-field detection by biosecurity surveillance personnel. The gram positive bacterium and select agent *R. toxicus* produces a toxin in annual ryegrass (*Lolium rigidum*). Livestock deaths in Australia due to toxin contaminated ryegrass have raised concerns about the global spread of *R. toxicus* in ryegrass hay and seed. Genomes from all available *R. toxicus* populations were explored to design target-specific loop-mediated isothermal amplification (LAMP) primers. Gene *rpoD* was selected for primers design. Internal LAMP primers targeting the ITS gene of host plant *L. rigidum* were also designed to enhance reliability and accuracy. Genie II, Sybr Green I and lateral flow device were used for LAMP assays. The developed assays were validated *in silico* against the NCBI GenBank nucleotide database and *in vitro* against the inclusivity and exclusivity panels for specificity. In-field assays were performed in South Australia; accurately detected *R. toxicus* from infected annual ryegrass samples. The developed

LAMP assays are sensitive, accurate and easy to implement. The proposed LAMP method will be helpful in diagnostics, surveys, disease management, and biosecurity decisions to support export/import of annual ryegrass.

#### **Development of recombinase-polymerase amplification assay for the detection of Western X phytoplasma ('*Candidatus Phytoplasma pruni*') in sweet cherry**

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Resurgence of Western X disease (WX) of sweet cherry (*P. avium*), caused by WX phytoplasma ('*Candidatus Phytoplasma pruni*'), has been observed in Washington State (WA) in recent years. The disease presents a major economic impact in WA sweet cherry production as affected trees not only decline but produce unmarketable small, insipid fruits. In this study, an isothermal recombinase-polymerase amplification assay (RPA) for the detection of WX phytoplasma was developed. Using sequence data generated by high-throughput sequencing of three isolates of WX phytoplasma from WA along with sequence available in the GenBank database, primers and probe were selected within the immunodominant protein (*idpA*) coding region. The RPA assay system has three attributes that make it a very useful tool for detection of WX phytoplasma by cherry growers: (1) it is fast and simple – results are obtained in 20 minutes and because it uses only crude sap preparation, no sophisticated equipment for nucleic acid extraction is necessary; (2) it is sensitive – the RPA assay showed similar sensitivity with PCR both in temporal detection throughout the growing season and the amount of bacterial DNA detected; and (3) it is specific only to WX phytoplasma. Additionally, RPA detected WX phytoplasma from total DNA extracts of three known leafhopper vectors of the pathogen; this makes it a valuable tool in pathogen vector research.

#### **Viability of *Candidatus Liberibacter asiaticus* in grapefruit leaves at different stages of maturity and Huanglongbing disease symptom development**

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Citrus Huanglongbing, associated with *Candidatus Liberibacter asiaticus* (CLAs), is the most serious disease in the USA causing substantial economic losses. The real-time PCR (qPCR) assays used to detect CLAs have a limitation that they cannot differentiate live and dead bacterial cells. Accurate quantification of live CLAs cells in the plant tissue is essential to devise effective disease management strategies and to test the efficacy of new disease control methods. Several optimized quantitative real-time PCR methods using propidium monoazide (VqPCR), a DNA-binding dye, to quantify live CLAs populations were described. However, it is unclear if live CLAs populations vary with age of the leaf and varying degrees of HLB disease symptom development. To address this question, we have quantified the levels of live CLAs populations in grapefruit leaves at different maturity stages and leaves with varying levels of symptoms collected from naturally infected field trees. VqPCR assay results obtained using two different chemistries including SYBR® and TaqMan® will be presented.

#### **Development of a multiplex real-time PCR for the improved detection of citrus canker-causing *Xanthomonas***

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To improve on the current assay for the detection of all citrus canker causing *Xanthomonas* which targets the *pthA* gene, a multiplex assay was developed to incorporate the 16S rRNA gene as a second target. Two new *pthA* amplicons designed to have better sensitivity than the current one, and two new 16S amplicons were evaluated individually, and in multiplex format. The PCR product of each amplicon was sequenced and verified to be the correct targets. Primers and probes interaction was determined and optimal concentrations selected followed by dNTP and MgCl<sub>2</sub> concentrations optimization for the different amplicon combinations. *In silico* and experimental specificity analyses were performed. All amplicons were found to have a 100% match with the target organism. The *pthA*-targeted amplicons showed cross-reactivity to some non-citrus *Xanthomonas* but not to other citrus-infecting genera. The method being developed targets symptomatic citrus therefore cross-reactions of the primers with *Xanthomonas* spp. of other crops should not interfere because most xanthomonads exhibit highly restricted host range (Delcourt et al., 2013). One of the 16S amplicons showed insufficient specificity thus was excluded from further optimization studies. Initial comparability studies suggest that the new *pthA* amplicons (used in multiplex with 16S rRNA gene) may be more sensitive than the currently used uniplex (*pthA*), having lower Ct values for the sets of archived diagnostic samples tested.

#### **Development of a rapid and reliable isothermal AmplifyRP diagnostic assay for specific detection of *Xylella fastidiosa***

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*Xylella fastidiosa*, infecting 309 plant species belonging to 63 families and 193 genera, is a regulated bacterial pathogen in many countries. Recently *X. fastidiosa* was associated with the devastating olive quick decline syndrome (OQDS) in southeastern Italy, resulting in an unprecedented concern to its spread in Mediterranean countries. In order to meet the needs for quarantine detection and field diagnostics, Agdia developed an advanced AmplifyRP assay for the detection of *X. fastidiosa*. The assay combined both endpoint (Acceler8) and real-time (XRT) detection in a single reaction PCR tube. This characteristic allows users to either collect the quantitative fluorescence data using a portable fluorometer or observe the qualitative test result in a disposable detection chamber. The assay is as sensitive as that of the published quantitative real-time PCR and has no cross-reaction to the closely related *Xanthomonas* species and numerous tested host plants including grape, citrus, and olive. The assay uses crude plant extracts and is applicable for use in both laboratories and fields. This assay provides users a fast and reliable tool to detect *X. fastidiosa* carried by plant materials and insect vectors from the contaminated regions.

#### **Recombinase polymerase-based diagnostics for in-field detection of *Pseudomonas syringae* pv. *actinidiae***

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In-field detection of the kiwifruit pathogen, *P. syringae* pv. *actinidiae* (Psa), will support plant biosecurity decisions regarding trade and production. To detect and discriminate among Psa strains (including the 2008 global outbreak strain, Psa-V), a Recombinase Polymerase Amplification (RPA) assay was developed for laboratory and field use. Primer sets targeting two effector genes (*hopZ3* and *hopZ5*) were designed and screened against isolates of highly virulent Psa and closely related *P. syringae* pathovars. All Psa strains can be detected by the presence of *hopZ3* amplicons but only the virulent type, Psa-V or biovar 3, is detectable by *hopZ5* amplicons. Because RPA assays were coupled to lateral flow device (LFD), reverse primer were labeled with either biotin or digoxigenin and the reaction was developed in the presence of a carboxyfluorescein (FAM) labeled probe. RPA assays had an amplification time of 10 minutes. Sensitivity determinations indicated a detection limit of 1 pg or 100 fg depending of the targeted gene. Isothermal assays are a rapid diagnostic assay that enables the early detection of pathogens and a valuable tool for in-field pathogen detection to support plant biosecurity decisions.

#### **Evidence for seed transmission of *Xylella fastidiosa* in pecan (*Carya illinoensis*)**

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*Xylella fastidiosa* is a gram-negative bacterial phytopathogen found exclusively in the xylem tissue of host plants. In pecan (*Carya illinoensis*), *X. fastidiosa* was previously shown to be the causal agent of pecan bacterial leaf scorch (PBLs) in the United States and was shown to result in yield losses and a reduction in overall plant health. We observed that *X. fastidiosa* in pecan is difficult to detect through PCR due to non-specific amplification and inhibition from phenolic compounds, additional efforts are being made to improve PCR diagnosis of this pathogen in pecan tissue and to determine the location of bacterial populations in infected trees. In previous studies on pecan, *X. fastidiosa* was presumed to be solely transmitted via xylem-feeding insect vectors such as sharpshooters and spittlebugs. However, in this study, PCR and sequence analysis identified the presence of *X. fastidiosa* in pecan seed from multiple cultivars. Currently, greenhouse studies are in progress to determine if *X. fastidiosa* in pecan seeds is viable and able to colonize seedling tissues. In addition, a microbiome enrichment kit will be used to identify microbial populations present in the samples using next generation sequencing (NGS).

#### **A computer program for fast and easy typing of *Ralstonia solanacearum* species complex strains into genospecies and sequevars 1&2**

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The phytopathogen *Ralstonia solanacearum* is a species complex that contains race 3 biovar 2 strains belonging to phylotype IIB sequevars 1 and 2 that are quarantined or select agent pathogens. Recently, the *R. solanacearum* species complex strains have been reclassified into three genospecies: *R. solanacearum*, *R. pseudosolanacearum* and *R. syzyzii*. An unidentified *R. solanacearum* strain is considered a select agent in the US until proven to be a non-race 3 biovar 2 (non-phylogroup IIB sequevars 1&2). We wrote a computer program that analyzes a minimum 400-bp user-input *egl* sequence from a *R. solanacearum* species complex strain for *egl* homology and SNP content to determine 1) whether it belongs to the *R. solanacearum* species complex, 2) if so, to which genospecies, and 3) whether it is of the sequevar type (sequevars 1 and 2) associated with the select agent/quarantined *R. solanacearum* strain. The program correctly typed all 371 tested *egl* sequences with known sequevars. It also successfully typed 25 *R. solanacearum* strains with no prior sequevar information, as well as 4 strains from infected plant samples, using their partial *egl* sequences amplified and sequenced with primers designed in this study. The *Ralstonia solanacearum* typing program does not require expertise or specific knowledge to use, gives results in seconds, and provides data interpretation for the user regarding the genospecies and sequevar 1 or 2 status of the strain in question.

#### **Real-time PCR detection and discrimination of the parsley pathogens *Pseudomonas syringae* pv. *apii* and *Pseudomonas syringae* pv. *coriandricola***

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Bacterial leaf spot in parsley is caused by two different *Pseudomonas syringae* pathovars, *Pseudomonas syringae* pv. *apii* and *Pseudomonas syringae* pv. *coriandricola* (Koike, Bull, 2011 Bull et al, 2011). Both were identified as causal agents in outbreaks of bacterial leaf spot in parsley. Seed was claimed to be the initial source of infection. Current seed test methods include a pathogenicity tests but lack confirmation methods. Therefore a realtime PCR assay was developed to detect and discriminate the two pathovars causing leaf spot disease in parsley. The primers and probes were developed on sequence variation in the gap1-gene of 35 closely related *Pseudomonas syringae* pathovars. Both P.s. *apii* and P.s. *coriandricola* were amplified with the forward primer 5'-ACCCAGTCGATGATCCCGA-3', reverse primer 5'-AGCGAAACGTTGATCACC GG-3', while probes differentiated P.s. *apii* (5'-6FAM-CAGGCAA CTGACCGGCATGCGCGTT-BHQ1-3') from P.s. *coriandricola* (5'-HEX-CGGGCAAGCTCACGGGGATGGCTGTT-BHQ1-3'). Primers and probe showed sufficient discriminative power at the genospecies level when tested on 52 closely or non-related strains. Primers were used in an improved seed health tests for the detection of P.s. *apii* and P.s. *coriandricola* on parsley seeds. The seed health test was validated and test results were confirmed for biological relevance using grow-outs of all used seed lots.

#### **PCR Assays for the Detection of *Xanthomonas euvesicatoria*, Causal Agent of Bacterial Spot on Pepper and Tomato Plants**

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*Xanthomonas euvesicatoria* (Xev) causes bacterial spot disease on pepper and tomato plants. A specific detection technique for this pathogen is important for the management of the disease and an efficient plant quarantine services. To obtain species-specific primers for PCR assays, low homologous ORFs of *Xanthomonas euvesicatoria* 85-10 to genes in NCBI GenBank were selected by BLASTn. Xev60 F/R primers that were designed from one of the select ORFs amplified specific DNA from all of tested strains of Xev including the 9 internationally collected strains and 67 Korean strains while the primers did not amplified any DNA from the 20 internationally collected strains of 3 other bacterial spot-causing *Xanthomonas*, *X. vesicatoria*, *X. perforans*, and *X. gadneri* and the other plant pathogenic bacteria. Taqman probe, Xev60-Tq was designed from the inside the PCR amplicon. Real-time PCR with Xev60 F/R and the Xev60-Tq probe generated Ct values with dose-dependent of target DNA ( ) and Ct values of Xev strains were above of threshold Ct value for positive detection. PCR assays developed in this study can reliably detect and identify Xev.

#### **Loop-mediated isothermal amplification for the detection of soft rot causing *Dickeya* spp.**

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*Dickeya* spp. cause soft rot, wilt and blackleg diseases in a wide range of plants and crops of agricultural importance. The emerging pathogen *D. solani* may be a contributing factor to the increased incidence of blackleg and soft rot diseases on potato in Europe, whereas *D. dianthicola*, a close relative, is currently listed on the European and Mediterranean Plant Protection Organization's list of pests recommended for regulation as quarantine pests. Presently, the North American Plant Protection Organization has placed *D. solani* on the U.S. list for emerging pest alerts. Symptoms caused by *Dickeya* spp. can be confused with those produced by *Pectobacterium* spp. in the field; differentiation of these two soft rot causing groups is time-consuming and requires multiple assays for confirmation. Therefore a robust rapid diagnostic test for the differentiation and detection of *Dickeya* spp. was developed using loop-mediated isothermal amplification (LAMP). In this study, a genus-specific LAMP assay was developed using whole genome data. The specificity of the assay was evaluated on six of the seven recognized *Dickeya* species and *Pectobacterium* spp. associated with potato; the LAMP assay only showed positive reactions with *Dickeya* spp. Direct detection of *Dickeya* from infected plant tissue was assessed and provided a result in less than 30 minutes. This LAMP assay offers a rapid and sensitive way for testing of *Dickeya* spp. from plant samples.

#### **Relative post-inoculation detection frequencies of 'Candidatus Liberibacter solanacearum' haplotypes in three solanaceous hosts**

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'Candidatus Liberibacter solanacearum' (Lso), vectored by the potato psyllid (*Bactericera cockerelli*), is a putative bacterial pathogen, which causes potato zebra chip disease. The pathogen also infects all known solanaceous plants including peppers and tomatoes. Currently there are two haplotypes (A and B) of the pathogen identified from potatoes, and individual psyllids can carry either A or B or both. Greenhouse experiments were conducted to investigate post-inoculation relative incidences of the haplotypes in host plants (pepper, potato, and tomato) and in successive psyllid generations reared on the hosts. Plants were infested with psyllids in 5 cages each (12 psyllids/cage) with each cage containing 2 plants. Psyllids were obtained from a

colony in which nearly 90% were positive for A+B (the rest B). Plant and psyllid samples from the cages were collected 3 times (about every 2 weeks) beginning 6 weeks after infestation and tested for Lso haplotypes using qPCR and SYBR analysis. There was a highly significant difference among the hosts in incidence of the haplotypes (Chisq=15.3, P<0.0001). In peppers, 70% of the haplotypes were A, and 30% A+B. Haplotype B by itself was not detected. In potatoes, 75, 18, and 7% of haplotypes were A+B, B, and A, respectively. However, nearly all samples from tomatoes (96.7%) had haplotype A+B (the rest B) but A as a single haplotype was not detected. Haplotype frequencies in psyllids followed a similar detection pattern.

#### **Early detection of *Leifsonia xyli* subsp. *xyli*, the causal agent of ratoon stunting disease, in sugarcane seedlings**

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The first major change of the Brazilian sugarcane industry over the last decades occurred when mechanical harvest replaced the manual cuttings of burned canes. The second transformation has started with mechanization of planting materials thanks to new methodologies such as pre-sprouted cane (PSC). To ensure the success of this innovation, however, is paramount the use of clean seed cane especially against ratoon stunting disease, caused by the fastidious bacterium *Leifsonia xyli* subsp. *xyli* (Lxx), which can only be spread over new areas by contaminated propagative materials. Therefore, the purpose of this work was to develop a routine diagnostic test for early detection of Lxx in PSC seedlings. In the first assay, four previously published pair of primers, Cxx ITS#5/Cxx ITS#5, Cxx1/Cxx2, C2F/C2R, LX23SF/LX23SR, was challenged under the original conditions, as well as, our own modifications against adult diseased sugarcane with various concentrations of Lxx. In the second assay, two of the best primers/conditions were tested against PSC of 45 and 60 days of age derived from propagative materials with different levels of Lxx ( $10^6$  to  $10^9$  UFC/ml). Our result showed primers Cxx ITS#5/Cxx ITS#5 run under our modified conditions was the most efficient since Lxx was detected in all 52 PSC seedlings of 45 days, including all 18 resulted from propagative materials contaminated with  $10^6$  UFC/ml. Part of MSc dissertation by the second author at PPGVBA, CAPES Scholarship.

#### **Development of a loop-mediated isothermal amplification assay for the detection of *Harpophora maydis***

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*Harpophora maydis*, a seedborne fungal plant pathogen that causes late wilt of maize, is an important pathogen that occurs in Africa, Europe and Asia, but not in the Americas. It is a high-priority quarantine pathogen listed on the APHIS-PPQ OPIS pest list. Thus, there is a need for effective pathogen detection assays that can be used to identify contaminated seeds. We have developed a loop-mediated isothermal amplification (LAMP) assay to detect *H. maydis* based on a specific AFLP sequence from GenBank. *H. maydis* isolates were grown on PDA and DNA was extracted with the Extract-N-Amp plant kit. A primer set (AFLP 17) consisting of 5 primers recognizing different regions of the target sequence was designed using Primer Explorer V4 software. The LAMP reactions were performed in a 25  $\mu$ l reaction mix (1X Isothermal master mix) and incubated at 67°C for 25 minutes. The amplified products were detected by fluorescence and results were confirmed by gel electrophoresis. The LAMP assay showed specificity to the pathogen as shown by negative amplification when tested with other fungal species and was developed without the need to standardize DNA concentrations. The assay was effective for detecting the pathogen in seed samples containing inoculated seeds. This LAMP assay should be suitable as a fast, easy to perform and reliable method to detect *H. maydis*, providing a tool to help prevent the introduction of this potentially devastating pathogen into the USA.

#### **Using Color Spectrophotometry to Evaluate Disease Severity of *Macrophomina phaseolina* in Soybean**

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Charcoal rot is a soilborne disease of soybean caused by the fungus *Macrophomina phaseolina* (Tassi) Goid. The fungus is associated with hot dry weather and can dramatically reduce yields. Two current methods are used to evaluate disease severity in root tissues: colony forming units (CFU) and visual severity ratings. These methods are time consuming and expensive. Therefore, a more efficient methodology is needed to improve the screening process. We evaluated the use of color spectrophotometry as an alternative method for screening disease severity in 2006-2010 using 340 conventional and 893 roundup ready cultivars. Field plants were sampled at the R7 growth stage and evaluated using CFU, visual ratings (1-5 scale), and a color spectrophotometer. As previously reported, CFU values and visual ratings were predictive of each other across all years ( $F=7.66$ ,  $p<0.05$ ). Color data was not predictive of severity ratings ( $F=2.70$ ,  $p=0.10$ ), however they were negatively correlated across all years ( $r=-0.62$ ,  $p<0.001$ ). This association suggests that this method may be used as alternative in disease severity quantification of charcoal rot.

#### **Detection of *Erysiphe necator* fungicide-resistant alleles in leaf and air samples using novel molecular diagnostic techniques**

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*Erysiphe necator* (*En*) the causal agent of grapevine powdery mildew is managed using fungicides such as quinone outside inhibitors (QoIs). Unfortunately, *En* is known to develop resistance to QoIs by a single nucleotide polymorphism, resulting in an amino acid substitution (G143A) in cytochrome b. To monitor this allele, a TaqMan assay was developed and validated against powdery mildew DNA from strawberry, blueberry, snap pea, squash, Gerbera daisy, rose, and oak plants. Single spored DNA isolates ( $n=103$ ) were used to verify the accuracy of the TaqMan assay by comparing it to previous trifloxystrobin germination tests. *In-vitro* tests of single spored isolates identified 20% as the necessary proportion of the mutant allele in a mixed DNA sample to test positive for resistance. Leaf and air samples from Oregon ( $n=130$ ) were utilized in field validations. Digital droplet PCR was also utilized to quantify the allele distribution in a mixed DNA sample and lowered the detection limit the mutant allele for positive resistance to <5% of the total *E. necator* DNA present. This study resulted in novel allele-specific TaqMan and Digital droplet PCR assays that detect G143A in *En*. This assay could be used to significantly reduce the labor required in dealing with leaf and air samples of *En* in the laboratory and could be used to more closely monitor resistance development for a standard management program of grapevine powdery mildew.

#### **Investigation of primary infection period of apple blotch and circular leaf spot based on loop-mediated isothermal amplification assay in Korea**

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Loop-mediated isothermal amplification (LAMP) method is widely used to detect various plant pathogens. In this study, we surveyed primary infection period of apple blotch (AB) on apple and circular leaf spot (CLS) on persimmon using developed LAMP method. The causal agent of AB and CLS; *Marssonina coronaria* and *Mycosphaerella nawae* have long incubation period to appear the typical symptoms. Up to now, primary infection period was predicted through conidia scattering period or measurement of number of conidia. To control the AB and CLS properly, primary infection periods should be clear. Following the *M. coronaria* and *M. nawae* have long incubation period, we developed two different LAMP assay which can apply to apple and persimmon, and surveyed primary infection periods of AB and CLS in Korea using developed LAMP assay. As the results, not only infection was confirmed in healthy apple and persimmon leaves, but also the infection periods were gradually earlier than before. Although the relationship between



primary infection period and fungicide program should be studied, it is assumed that, to control and prevent economical losses of AB and CLS, fungicide spray program should be change.

#### **A new paradigm for early diagnosis tool for major fungal disease in apple and persimmon**

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To diagnose the fungal disease, it can be diagnose when the visible symptoms or sign are appeared. Or molecular biological method, such as polymerase chain reaction (PCR) is widely used, however, it is hard to apply in field condition and takes long time. To overcome these problem, we applied optical coherence tomography (OCT) tool for diagnosis the major fungal disease; apple blotch (AB) and circular leaf spot (CLS) in apple and persimmon. The casual agents of AB and CLS is *Marssonina coronaria* and *Mycosphaerella nawae*, which has long incubation period in their host. Using these characteristics, we tried to find the differences between healthy and infected leaves in the incubation period stage. OCT is non-invasive method which can examine the leaf samples without treatment. A-scan analysis which showing signal intensity showed that there was difference between healthy and infected leaves. Furthermore, infected leaves which had any visible symptoms were appeared similar pattern of signal intensity with diseased leaves. And infection was re-confirmed using species-specific PCR assay. Based on these results, it is assumed that OCT can be a useful diagnostic tool for early diagnose of two major fungal disease in apple and persimmon.

#### **Immunochemical detection of the Silverleaf disease of fruit trees caused by *Chondrostereum purpureum***

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The Silverleaf disease affects numerous species, including important fruit crops such as apple, blueberry, cherry, kiwi, peach, pear and plum. The leaves silvering is caused by the accumulation of an enzyme (endoPG) produced by *Chondrostereum purpureum*, however, foliar symptoms are not visible until the third year after the orchard plantation. Curative control is not effective, thus, an early detection is essential to avoid the fungus dissemination. Therefore, the objective of this work was to develop an early and non destructive method to detect the disease. Using the endoPG's aminoacidic sequence, monoclonal antibodies were synthesized and DAS-ELISA protocol developed. Mature leaves showing different silvering intensity were assessed, using healthy plants as negative control and purified endoPG as positive control. The presence of *C. purpureum* in the plants was confirmed through its isolation on 25% potato dextrose agar (PDA) and by the amplification of the large intergenic spacer (IGS) región, located between 5S and 18S rDNA. Results indicated that there was a positive correlation between foliar symptoms intensity and endoPG concentration, and that the method was able to discriminate among leaves from healthy and diseased but asymptomatic plants. This detection method was validated in apple, blueberry, peach and plum, as far, becoming a useful tool to prevent the dissemination of the Silverleaf disease.

#### **Evaluation of molecular diagnostic methods for *Monilinia* species of regulatory concern**

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Brown rot caused by *Monilinia* species is an extremely destructive disease of pome and stone fruits in many producing areas of the world, resulting in significant pre- and post-harvest losses. Diagnostics of species is difficult due to overlapping morphology, host-ranges, and symptoms. There are currently no molecular diagnostics methods that clearly discriminate all closely related species. *Monilinia fruticola* and *M. laxa* are native to or established in the US. *Monilinia fructigena* and *M. polystroma* are widely distributed in Europe and Asia and are exotic for the US. Both are listed as level "A" pests of economic importance to US agriculture in the 2015 Prioritized Offshore Pest List and the 2016 Cooperative Agricultural Pest Survey (CAPS) List. *Monilinia mumecola* and *M. yunnanensis* have been described recently (in 2004 and 2011, respectively) but are of less known impact, and neither of these species is known from the United States. The objective of this study is to evaluate two existing conventional PCR assays for distinguishing between native and exotic *Monilinia* for regulatory purposes. We have verified analytical sensitivity and specificity of these assays and determined the functional sensitivity with field-derived or spiked samples. A two-stage molecular diagnostic protocol that incorporates both assays allows for discrimination among all six of these *Monilinia* species, and should facilitate the accurate diagnosis for CAPS surveys.

#### **Identification of genomics-based detection assay for *Rhizoctonia solani* anastomosis groups**

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*Rhizoctonia* is an important plant pathogen for many crops throughout the world causing plant tissue rot. Typically, diagnoses is accomplished using pure culture "tester" isolates and determining an anastomosis group. In order to develop new diagnostic tools, three mitochondrial genomes of *Rhizoctonia solani* were aligned with *Mauve* using the software Geneious 7.1. AG1 and AG3 had dramatically different mitochondrial genome sizes (160 Kb versus 250 Kb, respectively) due to the presence of introns. A region of the rRNA large subunit from the mitochondria was identified and several primers were designed. DNA was extracted from 5 isolates of *R. solani* followed by the polymerase chain reaction and sequencing. Sequence length for the large subunit of rRNA for each tested isolate varied significantly in size to what was expected. Difference in size is due to the presence and length of introns in the mitochondrial genome. Sequences were aligned for homology and differences. We are in the process of extracting purified genomic mitochondrial DNA from type isolates of *R. solani* to get a more complete picture of the significant diversity between AG groups. Even further research will be done to convert this method into a rapid field detection tool via recombinase polymerase amplification.

#### **Development, testing and pending deployment of a LAMP diagnostic screening method for the Citrus Black Spot pathogen, *Guignardia citricarpa***

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*Guignardia citricarpa*, the causal agent of Citrus Black Spot (CBS) was discovered in Florida during 2010. Today, three FL counties remain under quarantine. *G. citricarpa* causes unsightly lesions that reduce fruit marketability, and CBS is subject to national and international quarantine restrictions. The affected packing houses must monitor all fruits coming into the facility before they ship to the EU. If CBS symptoms are found on just a single fruit, entire shipments are put on hold where this perishable commodity's time is extremely valuable and a decision must be made. Current protocol requires that suspect fruit be shipped to PPQ or State Labs for confirmatory diagnosis which include a conventional and qPCR assay. We have developed a diagnostic tool that will enable inspectors to turn a 2-3 day decision into less than 1 hour using a LiNK extraction method followed by Loop-Mediated Isothermal AMplification (LAMP). The LiNK column allows for rapid DNA extraction for use on the Genie III, a durable, sensitive, and user-friendly isothermal platform. Our LAMP screening assay is able to detect down to 30 fg of DNA with amplification times between 5-20 minutes. The Genie has been able to reproduce qPCR results on all 55 citrus samples tested from Florida as well as 32/33 CBS DNA diagnostic samples submitted to CPHST. Further testing on environmental samples and non-target hosts are underway. We will discuss the advantages and limitations of the method and deployment.

#### **Rapid diagnostic tools for soilborne pathogens of strawberry**

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The ability to rapidly identify and quantify strawberry pathogens in the soil allows growers to make faster decisions about what to plant and how to manage diseases a field. Therefore, we are developing rapid assays using TaqMan real-time PCR and recombinase polymerase amplification (RPA) to diagnose a variety of pathogens commonly infecting strawberry fields, including *Verticillium dahliae*, *Fusarium oxysporum*, *Phytophthora* spp., and *Macrophomina phaseolina*. These assays can provide results in hours (TaqMan) or in as little as 20 minutes (RPA) using DNA extracted from either infected plant tissue or soil. Species-specific TaqMan and RPA assays have been developed for *Phytophthora* and are very sensitive (0.2 pg) and specific. For *V. dahliae*, a species-specific TaqMan assay with a sensitivity of 1-2 microsclerotia per gram of soil is being converted to an RPA assay. Host-specific markers for *F. oxysporum f. sp. fragariae* are currently being developed for both TaqMan and RPA using a locus previously published by Suga et al. (Plant Dis. 2013). Additional loci for *F. oxysporum f. sp. fragariae* are being identified through sequencing and comparative genomics of multiple isolates in order to expand the breadth of detection. For *M. phaseolina*, sequencing of multiple isolates has identified a genotype-specific locus, and primers are being developed for RPA and TaqMan assays to detect isolates which have been found to only infect strawberry.

#### **BioPCR: An improved method for detection of *Colletotrichum acutatum* in asymptomatic strawberry nursery plants**

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*Colletotrichum acutatum* infections in strawberry nurseries often stay symptomless. Infected plants usually collapse upon transplanting in grower fields resulting in economic losses to the growers. A routine method to detect *C. acutatum* in asymptomatic plants is to culture root and crown tissue on semi-selective agar media such as TPDA and BR and confirm identification by microscopic observations of spore morphology and PCR. In our experience, the culture method often fails to detect *C. acutatum* when non-target fungi such as *Trichoderma* sp., *Alternaria* sp. and *Aspergillus* sp. are present in a sample. The rapid growth of non-target fungi mask the pathogen in culture. To overcome this problem, we investigated BioPCR as an alternative method to detect infections in asymptomatic tissue. In BioPCR, tissue was cultured for four days on agar media to enrich *C. acutatum* in the plant tissue. After 4 days, tissue was removed from the culture plates and tested by PCR using primers specific for *C. acutatum* (1). Culture plates were still available for reading. During 2015, we analyzed 106 commercial samples from strawberry nurseries in California by culture and BioPCR method. BioPCR detected *C. acutatum* in 83% plants compared to 42% by culture method. We conclude that BioPCR method is superior to culture method and can be used to assay strawberry nursery plants for detection of *C. acutatum* in asymptomatic plants.

#### **Development of a quantitative PCR assay to quantify resistance to *Diaporthe helianthi* and *Diaporthe gulyae* in sunflower**

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In the U.S., Phomopsis stem canker was first detected in 1983, but it was not until the 2010 epidemic in Minnesota, North Dakota, and South Dakota that two pathogens, *Diaporthe helianthi* and *Diaporthe gulyae* were identified as the causal agents. At this time, no commercial hybrids are available that have complete resistance to the two pathogens. The objectives of this study were to (1) develop quantitative polymerase chain reaction (qPCR) assays for quantification of *D. helianthi* and *D. gulyae* from sunflower and (2) evaluate sunflower germplasm for resistance to the two pathogens using qPCR assays. The primers and probes for the two qPCR assays were designed from the translation elongation factor region for amplification of *D. helianthi* and *D. gulyae*. To evaluate sunflower germplasm for resistance to *Diaporthe* spp., 54 sunflower genotypes from 9 different countries were screened for resistance using the stem-wound method in the greenhouse. Disease severity was evaluated 14 days after inoculation using a 0-5 rating scale from Mathew et al. (2015). Preliminary results suggest significant differences among genotypes in their resistance to *D. helianthi* and *D. gulyae*; PI 561918 (HA 378) appeared to be less susceptible to the two pathogens than the susceptible check 'HA 288'. The qPCR assays developed in this study will be used to compare genotypes for resistance to *D. helianthi* and *D. gulyae* by quantifying the levels of pathogen DNA present in them after inoculation.

#### **Phylogenetic identification of pathogenic and endophytic fungal populations in west coast Douglas-fir foliage**

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Douglas-fir provides social, economic and ecological benefits in the Pacific Northwest (PNW). Minor defoliation has been linked to Swiss Needle Cast, associated with *Phaeocryptopus gaeumannii*. Unprecedented defoliation has increased since the 1990s, leading to decreased growth and yield. Affected areas exceed 500,000 acres in Oregon. Recent symptoms are inconsistent with predicted effects of *P. gaeumannii*. We hypothesize that the entire endophytic community is relevant to disease ecology, though this has not been investigated to date. Dynamics of needle cast are explored and baseline inventories of endophytes established by Sanger and Next-gen Sequencing (NGS). Endophytes were evaluated along environmental gradients at three sites in the PNW. Probability of occurrence (presence or absence of any endophyte in needle) was hypothesized to be higher in cooler, wetter climates. Preliminary results suggest that the probability of endophyte occurrence at the cool, wet site was 2.6 (p<0.0007) times higher than at the warm, dry site, and 3.7 (p<0.0001) times higher than at the warm, wet site. Despite advances in technology, few studies have identified endophytes in Douglas-fir by molecular means. Traditional culturing is hypothesized to be less effective than NGS. More inclusive identification could lead to greater efficiency in forest management. It is vital to understand the complete etiology of SNC and the ecological implications of disease in this important species.

#### **Rapid diagnostic assay development for detecting patch diseases on turf**

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Necrotic Ring Spot, Summer Patch, and Take-all Patch are turf root diseases caused by soil-borne plant pathogenic fungi. The infected turf shows aboveground symptoms that are broadly categorized as 'patch' diseases that ruin the aesthetic value of cool-season turfgrass on lawns/fields in North America from late spring to early summer. These fungal pathogens are notoriously difficult to isolate from roots. Therefore, we decided to develop a rapid detection assay that can be performed on suspected root samples. We are testing two assays: Loop mediated isothermal amplification (LAMP) and Recombinase polymerase amplification (RPA). Both LAMP and RPA do not require pure genomic DNA but only a solution of root sample. LAMP and RPA rely on DNA strand displacement activity for its rapid amplification and can be completed at 65°C in an hour and 39°C in 20 minutes incubation, respectively. Initial LAMP screening gave false positives repeatedly in almost all assays indicating that very pure conditions are needed, which is not ideal in a diagnostic laboratory. Hence, we have started to work on RPA methodology available as Agdia's discovery kit to eliminate any false positives. Once we achieve a consistent and specific amplification with these assays, we will proceed to screen samples that will be arriving in late Spring 2016. Assay development, primer-probe design and samples surveyed will be reported in this study.

#### **Rapid differentiation of *Claviceps* species occurring in Oregon and Washington using high resolution melting curve analysis**

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Ergot disease of grass seed crops can be caused by *Claviceps purpurea* and *C. humidiphila*, a species recently reported to occur in certain regions of OR and WA. Routine identification of *Claviceps* species involves conventional PCR amplification and sequencing of ITS,  $\beta$ -tubulin or EF-1 $\alpha$  genes. In this study, a high resolution melting (HRM) assay was developed to rapidly identify and differentiate *C. purpurea* and *C. humidiphila* based on the melting temperatures (T<sub>m</sub>) of partial  $\beta$ -tubulin amplicons. PCR primers were designed to target and amplify a 110 bp region of the  $\beta$ -tubulin gene containing multiple single nucleotide polymorphisms generating amplicons with distinct melting profiles for each species. Forty-six fungal cultures, previously classified based on RAPD profiles, and ITS,  $\beta$ -tubulin, and EF-1 $\alpha$  gene sequences, were used to validate the HRM assay. The T<sub>m</sub> for *C. purpurea* and *C. humidiphila* ranged between 79.8 to 80.3 and 78.9 to 79.3, respectively. Classification of *Claviceps* species based on T<sub>m</sub> using linear contrasts ( $P < 0.05$ ) correctly grouped 96% (N=46) of samples previously classified using sequence data. In two cases, *C. purpurea* was misclassified as a mixture of the two *Claviceps* species. Therefore HRM analysis allows rapid detection and differentiation of these two *Claviceps* species and will be useful to study their distributions within OR and WA cool-season grass seed growing regions.

#### **Specific detection of the root-lesion nematode *Pratylenchus scribneri* using conventional and real-time PCR**

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*Pratylenchus scribneri* is a plant-parasitic root-lesion nematode causing economic damage to various crops worldwide. Identifying root-lesion nematodes to species using traditional morphological method is an arduous task requiring extensive training on nematode taxonomy. Consequently, molecular methods were developed to identify *P. scribneri* by conventional and real-time PCR using a species-specific primer set that was designed based on the alignment of 20 sequences of internal transcribed spacer of rDNA of seven *Pratylenchus* spp. The primer set specifically amplified DNA of *P. scribneri* but not DNA from five other *Pratylenchus* spp. or ten non-*Pratylenchus* spp. A single melt curve was observed indicating a high specificity according to real-time PCR analysis. The capability of specific PCR was tested using DNA extracts of root-lesion nematodes from 23 soil samples collected from various fields with five crops in North Dakota and Minnesota. The PCR primers and protocols were successfully used in *P. scribneri* identification. The conventional PCR could detect DNA of 1:8 dilution of two nematodes equivalent to 1/4 of a nematode. The real-time PCR was more sensitive and able to amplify DNA of serial dilutions (2 nematodes - 1/128 nematode) with C<sub>q</sub> values ranging from 25.4±0.0 to 33.6±0.4. The developed PCR assays provide a rapid and reliable identification of *P. scribneri*, suitable for use in diagnostic laboratories for detection of field infestations with this nematode.

#### **Real-time and conventional PCR assays for identifying the stubby root nematode *Paratrichodorus allius***

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Stubby root nematode (SRN), *Paratrichodorus allius*, is an important pest on many crops, particularly, on potato due to its ability to transmit *Tabacco rattle virus* causing corky ringspot disease on tubers. Nematode surveys revealed that SRN is present in many fields on different crops in North Dakota and Minnesota. Detection and identification of SRN are important for effective disease management. Protocols of *P. allius* identification by real-time and conventional PCR with species-specific primers were developed. Primers were designed from internal transcribed spacer of rDNA based on sequences of *P. allius* and six closely related *Paratrichodorus* spp. A single amplicon (246 bp) was produced from DNA of *P. allius* only, but not from DNA of two other SRN species and 21 non-SRN species. Detection sensitivity analysis indicated that an equivalent of 1/20 of a nematode could be detected by conventional PCR and an equivalent of 1/1000 of a nematode by real-time PCR with C<sub>q</sub> value at 32.6±0.5. The PCR assays were tested with SRN from 20 soil samples collected from various fields with five crops in ND and MN, and they successfully amplified DNA from all samples. These SRNs were confirmed to be *P. allius* by sequencing of three genomic regions. The PCR assays are rapid and reliable and can be used in *P. allius* identification using nematode individuals. This finding will help develop a real-time PCR assay for quantification of *P. allius* directly from DNA extracts of field soils.

#### **Development of species-specific qPCR tests for detection and quantification of *Meloidogyne hapla***

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The Northern root-knot nematode, *Meloidogyne hapla*, is an important soilborne pathogen of potatoes and other vegetables grown in the United States and is common within New York State. Effective management of *M. hapla* and other plant-parasitic nematodes relies on the accurate identification and timely quantification of pathogen populations prior to planting. Identification and quantification may be substantially improved by pre-plant soil tests which utilize quantitative PCR (qPCR) techniques to target DNA sequences specific to the pathogen of interest. Here, qPCR primers were designed to target unique regions of an effector gene *16D10* in *M. hapla* and assayed for specificity against plant-parasitic nematodes using qualitative PCR. The primers developed in this study were shown to be specific to *M. hapla*. A qPCR tests was developed and the sensitivity of the primers determined.

#### **DNA-nanosensor based diagnostics of plant pathogens**

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Crop loss from plant pathogens is a leading threat to food security in the United States. Current pathogen detection assays have limited field operability, leading to improper disease management decisions. Recently, rapid diagnostic DNA-nanosensors have increased perception and aided precision care of human pathogens in resource limited locations. Here we demonstrate the application of DNA-based nanosensor to detect an obligate biotroph, *Pseudoperonospora cubensis*. The detection of *P. cubensis* is based upon primers highly specific to a targeted gene, and the physical properties of DNA and nanoparticles with sodium chloride. Diluted DNA was denatured and cooled in a solution with the primers. Then nanoparticles were added to the solution followed by sodium chloride to reveal primer-target interaction. Target DNA presence was quantified colorimetrically (i.e. naked eye) and by absorbance on a spectrophotometer over a 400 to 700 nm range. Current PCR amplified target can be detected to 5 pg/mL by the naked eye. Optimization of this DNA-nanosensor to detect extracted genomic DNA, and within environmental matrices will be discussed. The deployment of this rapid bioassay has the potential to assist growers in developing sustainable disease management decisions in a field based setting.

#### **Optimization of a fluorescence *in situ* hybridization assay for detection and visualization of *Plasmopara obducens* from plants and soil**

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*Plasmopara obducens* is the biotrophic oomycete responsible for downy mildew of ornamental impatiens, a destructive disease that significantly impacts the production of *Impatiens walleriana* in the United States. To date, there are no methods to detect *P. obducens* from asymptomatic plants, which may be contributing to the spread of the disease. Furthermore, the extent that *P. obducens* uses oospores to colonize and persist in the soil is undetermined. Fluorescence *in situ* hybridization (FISH) is a sensitive and robust method that uses sequence-specific, fluorescence-labeled oligonucleotide probes to detect target organisms from the environment. A FISH assay for the impatiens downy mildew pathogen was designed by preparing DNA sequence alignments of the rDNA-ITS region of *P. obducens* and other related taxa to identify suitable regions for species-specific probe development. Because *P. obducens* cannot be propagated *in vitro*, we developed a custom *E. coli* expression vector that transcribes the rDNA-ITS gene sequence of *P. obducens*

for use as a control and to allow for standardized fluorescence signal quantification (clone-FISH). The optimized probe hybridization stringency resulted in good fluorescence signal compared to those of the negative controls. The standardized protocol developed using the clone-FISH reagents will be used in subsequent experiments to directly visualize *P. obducens in situ* from plant and soil samples.

#### **Developing an innovative molecular toolbox for identification of *Phytophthora* species with emphasis on species of regulatory concern**

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The genus *Phytophthora* consists of 155 species, many of which cause significant economic impact to crops, ornamentals, and forest ecosystems worldwide. Many species are also exotic and of regulatory importance for the USA. Accurate species identification can be extremely challenging due to the need for extensive training and experience, as well as many misidentified DNA sequences deposited in public databases. In order to facilitate correct identification of *Phytophthora* species, we are pioneering work with international collaborators for the implementation of a “Molecular Toolbox” containing DNA sequences generated from the type specimens used for the original descriptions. Ten nuclear genes and five mitochondrial genes currently used for identification and phylogenetic studies have been evaluated. Six nuclear genes, including the YPT1 gene that is amplified with new primers developed by Schena, and one mitochondrial gene have been selected for use in our “Molecular Toolbox” based on their utility in the robust identification and phylogenetic studies of *Phytophthora* species. However, many of the types lack sequences for these different genes (over 50%) and predominantly only have ITS sequences available. We will provide all files generated from the DNA sequencing, which will also demonstrate the high quality of data generated, to be used for identification purposes. Emphasis of this project is for species of high economic impact and regulatory concern for the USA.

#### **Evaluation of Recombinase Polymerase Amplification (RPA) Isothermal Amplification Diagnostic Assay for *Phytophthora ramorum***

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*Phytophthora ramorum* was found as the causal agent of ‘Sudden Oak Death’ oaks and tanoaks in the coastal counties of California in the 1990s. Since then, the pathogen has been confirmed on numerous native hosts and nursery plants in California, Oregon, and Washington, with sporadic occurrences in other regions of the USA. The current methods of testing for *P. ramorum* include conventional and real-time PCR which are expensive and timely. The primary purpose of this work is to enhance Plant Protection and Quarantine efforts in the detection of *P. ramorum* by evaluating a field-deployable, simple, and rapid detection method. A previously published Recombinase Polymerase Amplification (RPA) Isothermal Amplification Assay for detection of *P. ramorum* was evaluated for both sensitivity and specificity using the Twista™ apparatus. DNA extracted from pure culture, infected and healthy rhododendron tissue, as well as environmental samples were used to validate the *P. ramorum* protocol for regulatory use. Preliminary data comparing the isothermal amplification assay to current confirmatory *P. ramorum* diagnostic assays, evaluating ITS and Elicitin qPCRs shows that this protocol was not as sensitive as the ITS qPCR, but was comparable to the Elicitin qPCR. We will present the value of RPA isothermal amplification as a regulatory diagnostic tool for the rapid detection of *P. ramorum*.

#### **Extracting DNA for qPCR amplification from plant tissue in ELISA buffer**

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USDA APHIS mandates testing protocols for *Phytophthora ramorum*. Diagnostic labs use an ELISA kit to screen for *Phytophthora* infection in leaves. If a leaf tests ELISA-positive, a second sample is collected from the leaf for DNA extraction and molecular testing. This may delay detection by a day. DNA extraction directly from macerated leaf tissue in ELISA buffer would speed testing. To determine if DNA extracted from such tissue would be adequate for molecular testing, 198 leaf samples from a nursery were tested with ELISA, with 30 testing positive. For each ELISA-positive sample, leaf tissue was collected and DNA extracted following WI-B-T-2-3 Rev. 1; DNA was also extracted from the macerated leaf tissue in ELISA buffer. Tissue-DNA and ELISA-DNA was then tested for *P. ramorum* following N-R-WI-008-0 using the reporter dyes FAM (*P. ramorum*-specific) and JOE (internal control); all DNA was tested undiluted and diluted 1:10. For all samples, the tissue-DNA and ELISA-DNA tested negative for *P. ramorum* (FAM Cq = 0.00) at both dilutions. For the internal control, the tissue-DNA tested positive for amplifiable DNA with a mean Cq of 27.30, whereas the ELISA-DNA tested positive with a mean Cq of 26.41. For diluted DNA, the mean Cq for tissue-DNA was 20.45 and for ELISA-DNA was 20.79. The results suggest amplification of the internal control was comparable between DNA extracted from leaf tissue and DNA extracted from macerated leaf tissue in ELISA buffer.

#### **Utilizing mitochondrial loci to develop TaqMan and recombinase polymerase amplification assays for the genus *Pythium***

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Mitochondrial marker systems have been utilized in other oomycete systems to develop novel molecular diagnostic tools in both laboratory and field settings. For *Pythium* a mitochondrial gene order difference was identified that allowed for genus specific amplification of more than 75 species across a wide range of clades within the genus. PCR results for this locus tested negative on *Phytophthora* and several *Phytophythium* spp. A TaqMan diagnostic assay was developed using a FAM labeled probe and validated on environmental samples from over 40 host plants (nursery and field samples). A sequencing protocol was also developed to confirm a positive, however, if multiple species are present amplicon cloning will be needed to get clean data. In addition to the TaqMan assay an assay using the isothermal technology known as recombinase polymerase amplification (RPA) was developed. Using the same purified and field samples the RPA technique was effective at detecting *Pythium* species in less than 20 minutes without a laborious DNA extraction. The use of this marker system will not only help confirm *Pythium* infection in plants, it may also be useful in environmental samples and it will complement the other marker system developed for *Phytophthora*. Sequencing of additional species is in progress to evaluate species specific detection/identification capabilities. Due to the amplicon size, this locus may also be useful in future metagenomic studies of *Pythium* diversity.

#### **Development of molecular diagnostic tools for the invasive oomycete pathogen *Phytophthora tentaculata***

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*Phytophthora* basal rot (PBR) of plants is a disease recently observed in Central California Native plant nurseries and restoration locations. The primary causal agent of this disease is the oomycete pathogen *Phytophthora tentaculata*. Prior to 2012, *P. tentaculata* was among the ~30 *Phytophthora* species not known to be present in the US. Previous research had developed an isothermal diagnostic tool known as recombinase polymerase amplification (RPA), which had *Phytophthora* genus specific detection capability with results obtainable within as little as 15 minutes directly in the field without conventional DNA extraction. A *P. tentaculata* species-specific RPA assay was developed and specificity validated against pure DNA from 135 *Phytophthora* taxa. To test this technique for detection of the pathogen in PBR, 113 symptomatic samples were collected and evaluated with the new RPA assay as well as the previously validated TaqMan assay, Immunostrip and conventional culturing and baiting techniques. Results were similar across the various amplification platforms, with qPCR being the most sensitive in general. Finally, spatial models were created to test if elevation correlated with the presence of the pathogen. These results indicate that *P. tentaculata* is often present in low-lying areas when infected plant material is used for restoration. This information will assist in more efficient, rapid, specific pathogen detection for management decisions in a field.

### **A CANARY Assay for *Phytophthora* spp.**

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*Phytophthora* is a genus of Oomycetes (water molds) that can cause serious plant diseases and considerable economic loss. A notable example is *P. infestans*, which was responsible for the potato late blight that caused the Great Irish Famine in the 1840s, and is still the most destructive pathogen of solanaceous crops. Another example is *P. ramorum*, the cause of Sudden Oak Death which has had devastating effects on forests in California and Oregon. Efficient management of *Phytophthora* diseases requires early detection and accurate identification of the pathogen. CANARY<sup>®</sup> (Cellular Analysis and Notification of Antigen Risks and Yields) is a fast, sensitive, and easy to use technology that has been successfully utilized for the detection of bacteria, viruses, and toxins. We are developing a CANARY<sup>®</sup> assay for *Phytophthora* spp. Using pure culture preparations, the assay was able to detect all species of *Phytophthora* tested, including *P. cactorum*, *P. cambivora*, *P. capsici*, *P. cinnamomi*, *P. citrophthora*, *P. kernoviae*, *P. infestans*, *P. nicotianae*, *P. niederhauserii*, *P. ramorum*, and *P. sojae*. Importantly, exclusivity studies showed that the assay did not cross-react with *Pythium* spp. Preliminary testing with infected plant material indicates that the assay takes less than 20 minutes to complete. A user-friendly software interface guides the user through all steps of the assay, providing a simple and rapid screening method for *Phytophthora* spp.

### **Early detection and quantification of *Pseudoperonospora cubensis* airborne sporangia using real-time PCR**

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*Pseudoperonospora cubensis*, the causal agent of cucurbit downy mildew, recently reemerged as a major destructive pathogen of a broad range of crops belonging to the Cucurbitaceae. Airborne sporangia of *P. cubensis* are the most important source of inoculum to initiate disease as well as cause secondary plant infections. Furthermore, airborne sporangia of this obligate oomycete are known to be able to travel long distances and survive for prolonged periods of time to solar radiation. Therefore, early and rapid detection of the airborne sporangia could serve as a warning for potential disease outbreaks. By designing genome specific primers and using SYBR green-based qPCR method, a preliminary threshold detection level of target pathogen DNA was determined in extracted DNA from *P. cubensis* sporangia and infected plant tissue. While the method successfully detected up to 5.3 pg/ml of sporangia DNA, the minimum DNA concentration required from infected plant material for successful detection was found to be almost 10-fold higher (69 pg/ml). Using Next Generation Sequencing (NGS) and bioinformatics tools, several unique and conserved candidate genomic regions were also generated to serve as diagnostic genomic markers for *P. cubensis*. These candidate genomic regions were tested for their suitability as diagnostic markers for detecting and monitoring airborne *P. cubensis* sporangia inoculum level in the field using real-time qPCR following entrapment with spore traps.

### **On-site detection of *Pythium ultimum* in potatoes using loop-mediated isothermal amplification (LAMP)**

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*Pythium ultimum* is a soil-borne plant pathogen that causes damping off and root rot diseases in a wide range of crops. In potatoes, *Pythium* leak caused by *P. ultimum* has been a major storage disease problem in the Pacific Northwest potato production areas for many years, but in recent years reported losses have increased. Since similar symptoms can be caused by other pathogens, rapid and robust tests are required to detect the pathogen. Loop-mediated isothermal amplification (LAMP) has great potential for diagnostic use offering fast, specific and sensitive detection. In this study a new LAMP assay was designed for *P. ultimum* and evaluated in terms of specificity and sensitivity. The assay was highly specific when tested with DNA from 30 other isolates consisting of closely related *Pythium* species and other potato pathogens. The assay was also highly sensitive detecting as little as 17.5 fg of DNA in less than 30 minutes when used with a Genie<sup>®</sup> II for fluorescence measurement. A simple, rapid on site DNA extraction method for tubers was developed for use with the assay which resulted in a highly sensitive test capable of detecting the pathogen in range of both naturally infected asymptomatic and symptomatic tubers. In conclusion, LAMP assays can offer a rapid, sensitive, on-site test for the presence of *P. ultimum* in potato tubers.

### **Sensitive detection and discrimination of HPWMoV, WSMV and TriMV using multiplex RT-PCR and High Resolution Melting: Part II**

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*High Plains wheat mosaic virus* (HPWMoV), *Wheat streak mosaic virus* (WSMV) and *Triticum mosaic virus* (TriMV) cause disease in wheat crops in Central and Western USA, including Oklahoma and Kansas. Although these viruses are from different families, they are transmitted by *Aceria tosichella*, the wheat curl mite (Prostigmata: Eriophyidae). These viruses may infect simultaneously single plants. Melting curve analysis of RT-PCR products obtained by High Resolution Melting (HRM) enables to detect and to discriminate different virus species. The method is rapid, sensitive and allows discrimination among multiple samples. Specific primers for original single endpoint RT-PCR and reference positive controls from Agdia<sup>®</sup> were used as previously reported (Part I this research, 2013) to generate cDNA. uMELT<sup>SM</sup> analysis of expected PCR products was used to predict HRM scenarios. Special attention was given to PCR product size, GC percentage and melting temperature (T<sub>m</sub>). T<sub>m</sub> derived from dF/dT plots *in vitro* were similar to predictions by uMELT<sup>SM</sup> *in silico*. All primers sets showed a sensitivity down to 1 fg/μL from cDNA in single-endpoint RT-PCR reactions. HRM assays are sensitive and allows detection and discrimination of viruses causing diseases and significant economic losses in wheat and corn.

### **Detection of *Apple scar skin viroid* in apple fruits by reverse transcription loop-mediated isothermal amplification (RT-LAMP)**

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*Apple scar skin viroid* (ASSVd) is one of the most important pathogen causing scar skin, dappling or cracking on the surface of apple fruits. A reverse transcription loop-mediated isothermal amplification (RT-LAMP) assay was developed for detection of ASSVd. A set of two primers were designed inner primers (FIP and BIP) and outer primers (F3 and B3) from sequences of the Korean strain of ASSVd. The LAMP reaction was optimized using *Bst* DNA polymerase for 30 min at 65°C. The RT-LAMP products were evaluated as ladder-like bands by electrophoresis and visualized in the reaction tube by staining with SYBR Green I dye. RT-LAMP was more sensitive than that of conventional RT-PCR for the detection of ASSVd. The RT-LAMP assay developed in this study is a simple, rapid and sensitive and can be applied as a practical molecular diagnostic tool.

### **Three genomic regions of *Cilevirus* allows detection and discrimination of two related species infecting citrus**

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Citrus leprosis disease (CLD) is caused by diverse etiological agents such as *Citrus leprosis virus C* (CiLV-C) and a newly proposed Citrus leprosis virus cytoplasmic type 2 (CiLV-C2), both in the genus *Cilevirus* and transmitted by Tenuipalpidae (false spider mites) *Brevipalpus* spp. CLD is also caused by Citrus leprosis virus nuclear type (CiLV-N) a possible *Dichorhavirus* (*Dichorhabdovirus*) and Hibiscus green spot virus 2 (HGSV 2) a *Higrevirus*

infecting citrus trees in Hawaii. The presence of CiLV-C in the western plains of Colombia was reported in 2005 using reverse transcription polymerase chain reaction (RT-PCR). A second report of CiLV-C2 sequenced from symptomatic citrus samples using next generation sequencing was made in 2013. These two reports had raised questions about distribution of CiLV-C and CiLV-C2 in Colombia and whether both viruses coinfect citrus groves. This study analyzed genomic sequences of CiLV-C and CiLV-C2 seeking target sequences for detection and discrimination using reverse transcription multiplex PCR. The developed assay is fast, sensitive and specific for detection and discrimination of these two species of *Cilevirus* infecting citrus. Only CiLV-C2 and no coinfections were found in samples collected in the citrus growing region of Meta and Casanare, Colombia.

#### **Development of conventional and Real-time RT-PCR assays for *Strawberry necrotic shock virus***

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*Strawberry necrotic shock virus* (SNSV) is one of the several viruses reported in strawberry and *Rubus* as a component of viral complexes that cause plant decline. Highly sensitive conventional and real-time RT-PCR detection assays were developed; able to detect all sequenced isolates of the virus. Primers and probes were designed from conserved regions of the virus coat protein gene and reactions were optimized to ensure both specificity and sensitivity. Comparison between the two assays revealed an at-least 100-fold higher sensitivity for the latter. Both tests were successfully validated using samples collected from several areas in the United States as well as a collection of isolates from around the world. These assays could be integrated into virus screening programs in certification, nursery or commercial field settings in both strawberry and *Rubus*.

#### **Rose rosette virus detection using loop-mediated isothermal amplification (LAMP)**

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*Rose rosette virus* (RRV) is an *Emaravirus* transmitted by *Phyllocoptes fructiphilus* strongly associated with Rose rosette disease (RRD). Visual diagnosis maybe confusing and specific, sensitive, easy to use with visual detection methods are required. LAMP is an isothermal amplification that combines these criteria. Alignment of 20 RRV P4 (RNA 4) accessions allowed primer design seeking broad detection of reported isolates. Optimal amplification was obtained with Bst 2.0 WarmStart<sup>®</sup> DNA polymerase (0.32 u), outer primers F3 and B3 (0.2  $\mu$ M), internal primers FIP and BIP (0.8  $\mu$ M) and MgSO<sub>4</sub> (4 mM) at 66.5°C for 1 hour. Bovine serum albumin (BSA) (4 mg/mL) and polyvinylpyrrolidone (PVP) (1%) were added as isothermal amplification enhancers. The detection limit was 1 pg/ $\mu$ L with plasmid carrying the targeted sequence. Products were visualized by electrophoresis. The visual detection limit of plasmid in colorimetric reactions using Hydroxynaphthol blue (HNB) (120  $\mu$ M) without BSA and PVP was 0.01 ng/ $\mu$ L. No cross-reactions with cDNA from ten frequently rose co-infecting or related viruses (HPV, MSV, INSV, TSWV, GRSV, ArMV, PNRSV, TRSV and TMV) was detected. Healthy tissue and non-template controls were included in all reactions. RRV-LAMP tested successfully with 25 tissue samples of symptomatic RRD roses from Oklahoma. The method has potential applications in biosecurity, microbial forensics and nursery virus-free monitoring of germplasm.

#### **Development of a field-based detection technique for *Rose rosette virus* using isothermal reverse transcription-recombinase polymerase amplification**

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Rose rosette disease, caused by *Rose rosette virus* (genus *Emaravirus*) is a major threat to the rose industry in the U.S. The only strategy currently available for disease management is early identification and eradication of the infected plants, thereby limiting its potential spread. Current RT-PCR based diagnostic methods for RRV are time consuming and is inconsistent in detecting the virus even from symptomatic plants. Hence a novel probe-based isothermal reverse transcription-recombinase polymerase amplification (RT-exoRPA) assay, based on the nucleocapsid gene of the RRV, has been developed. The assay is highly specific as tested against other common rose infecting viruses (both inclusive and exclusive). Dilution assays using the in vitro transcript showed that the assay is highly sensitive, with a detection limit of 1 fg. Moreover a rapid technique for the extraction of viral RNA (< 5 min) has been standardized from RRV infected tissue sources, using a single buffer which facilitates virus adsorption, followed by denaturation to release the RNA. RT-exoRPA analysis of the infected plants indicated that the virus could be detected from leaves, stems, petals, primary roots and secondary roots. The entire process including the extraction can be completed in less than 15 min, with less sophisticated equipments. The developed assay is currently evaluated for efficiency in large scale field testing for rapid detection of RRV in commercial nurseries and landscape.

#### **Development and characterization of polyclonal and monoclonal antibodies to *Rose rosette virus***

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Garden roses, which form the cornerstone of the multi-billion dollar landscape industry, annually generate wholesale US domestic production valued at ~\$400 million. Over the past few decades Rose rosette disease, caused by *Rose rosette virus* (RRV; genus *Emaravirus*), has become a major threat to the rose industry in the U.S. The only strategy currently available for disease management is early identification and eradication of the infected plants, thereby limiting its potential spread. Key to this effort is the development of efficient diagnostic tools to enable sensitive, rapid, user-friendly and accurate detection of the virus; currently limited to nucleic acid-based methods, such as PCR. With the aim of developing a serological diagnostic tool, rabbit polyclonal and several mouse monoclonal antibodies have been developed to the nucleocapsid protein (NP) of RRV. These antibodies were evaluated against NP peptides, cloned NP expressed in bacteria and plants, and RRV-infected plants. These results and those using the antibodies in various serological assay formats, including double- and triple-antibody sandwich ELISA and membrane-based assays, for lab and field detection of *Rose rosette virus* in infected sources, will be presented.

#### **Validation of RT-PCR High Resolution Melting as a detection method of *Tombusvirus***

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Plant waterborne viruses, such as some in the genus *Tombusvirus*, spread as colloids in water and also by mechanical transmission. Broad detection and simultaneous species discrimination of viruses in this genus is needed for testing field samples. RT-PCR coupled to High Resolution Melting (HRM) has been used for different forensic applications and allows species differentiation as well as SNP detection. *Tombusvirus*. complete genomic sequences retrieved from the NCBI-Genbank were used to design primers for broad detection using this method. Ten different virus species, *Carnation italian ringspot virus* (CIRV), *Cucumber necrosis virus* (CNV), *Cymbidium ringspot virus* (CymRSV), *Grapevine Algerian latent virus* (GALV), *Neckar river virus* (NRV), *Pelargonium leaf curl virus* (PLCV), *Pelargonium necrotic spot virus* (PNSV), *Petunia asteroid mosaic virus* (PetAMV), *Tomato bushy stunt virus* (TBSV) and *Pelargonium line pattern virus* (PLPV) were sourced by the DSMZ (Germany) and AGDIA (USA) and used for nucleic acid extraction. All viruses tested positive to broad detection endpoint RT-PCR with primers designed to react when coupled to HRM. Melting curve predictions calculated by uMelt<sup>SM</sup> algorithms were compared with data obtained using three DNA dyes (SYBR<sup>®</sup> Green, SYTO<sup>®</sup>9 and LCGreen<sup>®</sup>). Results with LCGreen<sup>®</sup> are closer to the predicted data calculated by uMelt<sup>SM</sup> when the amplicon size is between 50-200bp.

### **Virus detection of *Tobamovirus* with wide spectrum degenerate oligonucleotides by TD RT-PCR High Resolution Melting**

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Tobamoviruses cause notable plant diseases worldwide in *Solanaceae*, *Cucurbitaceae*, orchids and some are reported to be waterborne. Molecular tools exist for detection of this genus, however, a detection method combining genus detection and species discrimination is needed. Eleven reference positive controls from the DMSZ repository (Germany) and AGDIA (USA), were studied. Complete *Tobamovirus* genomes were aligned and a single antisense primer was designed to target the RNA dependent RNA polymerase (RdRp). This antisense primer co-reacted with five sense primers for selective subgroup detection by RT-PCR and HRM discrimination of eleven species within five subgroups. A protocol for detection and discrimination based on Touchdown Reverse transcription-Polymerase Chain Reaction (TD RT-PCR) coupled to High Resolution Melting (HRM) was developed. Species specific  $dF/dT$  curves ranged 75-85°C and were generated for each primer groups as follow: I (BpEMV, PMMoV, TMV, ToMV), II (PaMMV, TMGMV), III (SFBV, RMV), IV (ORSV, CGMMV) and V (SHMV). The method allows the incorporation of new groups of tobamoviruses to the assay by designing additional group specific sense primers. The resulting 11 diagnostic sequences showed 98-100% identity regarding the targeted viruses. HRM facilitates a reliable discrimination of species specific profiles for each virus species. The method is amenable to microbial forensics diagnosis and biosecurity applications.

### **Discriminating *Potexvirus* species by RT-PCR coupled to High Resolution Melting**

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*Potexvirus* is a cosmopolitan plant virus genus and most of its thirty-seven confirmed species are mechanically transmitted, causing mosaic and ringspot symptoms on herbaceous hosts. High Resolution Melting analysis (HRM) allows the discrimination at species and strain levels and has application in diagnostics. Complete genome sequences for *Alternanthera mosaic virus* (AltMV), *Clover yellow mosaic virus* (CIYMV), *Lettuce virus X* (LeVX), *Opuntia virus X* (OpVX), *Papaya mosaic virus* (PapMV), *Pepino mosaic virus* (PepMV), *Potato aucuba mosaic virus* (PAMV), *Potato virus X* (PVX), *Schlumbergera virus X* (SchVX), *Strawberry mild yellow edge virus* (SMYEV) and *Hosta virus X* (HVX), were retrieved from NCBI Genebank and aligned using Mega 6 and screened for conserved domains. Primer set Potex F3/R3 was selected to discriminate among eleven potexviruses by a melting temperature prediction using uMelt<sup>SM</sup>, a melting curve prediction software program. Reference positive controls for these eleven viruses were obtained from DMSZ (Germany) and Agdia, Inc. (USA) and tested using RT-PCR coupled to HRM with Potex F3/R3. All viruses tested positive and showed different melting temperatures ranging from 77.9°C to 82.4°C allowing for their discrimination through HRM analysis. The combination of RT-PCR-HRM can be used in diagnostic laboratories to discriminate eleven *Potexviruses* with high confidence.

### **A preemptive detection system to screen water for the presence of plant viruses**

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Growers are expanding cultivation into non-favorable cropping areas pumping water from nearby lakes, rivers, wells, aquifers, and runoff, increasing the need for waterborne plant pathogen monitoring. Particulate matter dilution factors due to high water volumes, make detection and identification of potential pathogens a challenge because of the extremely low virion numbers. Current plant pathogen detection methods are passive, and sampling and testing occurs after disease symptoms appear. In the event of highly virulent pathogen incursions, detection may be too late to limit losses. This project seeks to develop a preemptive detection system that will easily sample large volumes of water to screen for the presence of plant pathogens. Three waterborne plant viruses: *Tomato bushy stunt virus*, *Pepino mosaic virus*, and *Pepper mild mottle virus* were used as models for this purpose. Previously developed multiplex detection protocols, were modified to detect these three waterborne viruses. Recovery of viruses using polyethylene glycol from 250 ml, 500 ml, and 1 L of water was demonstrated. Adaptation of an inexpensive, durable, and field-ready sampling device is presented. The testing of waterborne virus movement in two different microcosms is described. Monitoring of microbial loads in agricultural irrigation systems, and other water sources, is essential for effective surveillance and disease prevention for plant health.

### **Multiplex RT-PCR detection of eight viruses infecting cucurbits and discrimination using High Resolution Melting analysis**

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Cucurbits are one of the largest horticultural commodities in United States and Oklahoma largely contributes with watermelon production. However, the cucurbit cultivated acreage of Oklahoma had decreased, in part, due to viral infections. This research targets eight disease-causing viruses: *Cucumber mosaic virus* (CMVI and CMVII), *Cucurbit yellow stunting disorder virus* (CYSDV), *Melon necrotic spot virus* (MNSV), *Papaya ringspot virus* (PRSV), *Squash mosaic virus* (SqMV), *Watermelon mosaic virus* (WMV), and *Zucchini yellow mosaic virus* (ZYMV). A rapid detection and discrimination method is needed for biosecurity and breeding for resistance against these viruses. Sequences from the polymerase protein gene (RNA) from the six genera were retrieved from NCBI and aligned using MEGA 6. Primer sets were designed from conserved regions using the web application PrimerQuest<sup>®</sup> and the thermodynamic features of each primer were tested using Oligoanalyzer 3.1. A Multiplex RT-PCR was developed to be coupled to High Resolution Melting (HRM) analysis for rapid detection of the targeted viruses. The obtained melting profiles allowed a clear discrimination among these viruses. This Multiplex RT-PCR High Resolution Melting method has potential to be applied in epidemiological studies of viral diseases in cucurbit crops, microbial forensics and biosecurity.

### **Toward broad detection of emaraviruses: Endpoint RT-PCR**

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*Emaravirus* has six confirmed species and two unclassified species. Rapid detection and an effective screening of existing and new emaraviruses is needed for detection, identification, plant virus discovery and biosecurity applications. Available sequences of RNA dependent RNA polymerase (RNA 1) for *European mountain ash ringspot-associated virus* (EMARaV), *Fig mosaic virus* (FMV), *Pigeonpea sterility mosaic virus* (PPSMV), *Pigeonpea sterility mosaic virus 2* (PPSMV2), *Rose rosette virus* (RRV), *High Plains wheat mosaic virus* (HPWMoV) formerly High plains virus (HPV), *Raspberry leaf blotch virus* (RLBV) and *Redbud yellow ringspot-associated virus* (RYRSaV), were aligned using Mega 6. A primer set (EMARA F&R7/8) was designed from conserved domains after strand sense normalization. The sensitivity of EMARA F&R7/8 was found to be 100 fg/reaction assessed using a reference positive control of HPWMoV (Agdia). The multiple *Emaravirus* detection was confirmed with three rose samples showing symptoms caused by RRV from three locations in Stillwater, OK, a HPWMoV reference positive control, and cDNA of EMARaV and FMV. All samples were screened by endpoint RT-PCR and tested positive for HPWMoV, RRV, EMARaV and FMV. Other emaraviruses (RLBV, RYRSaV, PPSMV and PPSMV2) were detected *in-silico* using Primer-Blast. This method addresses the need for sensitive molecular detection methods and discovery of novel emaraviruses.

### **Waterborne plant virus sampling and detection in aqueous environment**

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The aim of this research studying water sampling as a critical tool for detection of unwanted plant pathogenic virus that can seriously damage agriculture and environment. Water sources, such as reservoirs, lakes, farm ponds, tanks, and irrigation systems are vulnerable to inadvertent (most frequent) and intentional (rare) contamination by microbial pathogens. Pathogen detection in large bodies of water is complex because volume, dilution and water dynamics. This project assesses the development of a method for water sampling and detection of waterborne plant viruses of concern for water-security. Rapid detection and monitoring of high consequence plant pathogens in water systems, where may be present at extremely low titers present a significant challenge. The project seeks statistically validation of a water sampling model that uses quantitative-PCR as the method to detected *Potexvirus*, a genera of plant virus reported to have water borne members. In order to determine the minimum amount detectable in water, sensitivity tests will be conducted by ELISA and qPCR. Different pH will be tested during a process of virus filtration using a filtration system based on glass-wool. The capture is based on the isoelectric point affinity of the virus capsid protein with glass wool at an acid-neutral pH. This research is needed to support decision-making and will be useful for survey analysis and prevention, detection, and mitigation of unwanted waterborne plant viruses.

#### **Occurrence of endornaviruses in non-cultivated plant species**

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Large dsRNAs have been extracted from plants infected with acute and persistent viruses and from fungi infected with mycoviruses. These extracted dsRNAs have been used for viral disease diagnosis and virus identification. We used a modified version of the non-phenol batch method, in combination with reverse-transcription PCR (RT-PCR), to test symptomless, non-cultivated, plant species for endornaviruses. Foliar tissues were collected from different plant species from various locations within the city of Baton Rouge, Louisiana. Tissues were desiccated with silica gel and used for dsRNA extractions. After testing 132 plant species, belonging to 60 families, six yielded dsRNAs similar in size (14-15 kb) to reported endornaviruses. Putative endornaviruses were detected in *Alternanthera philoxeroides* (alligator weed), *Geranium carolinianum* (wild geranium), *Sonchus asper* (spiny sow thistle), *Sorghum halepense* (Johnson grass), and *Hydrocotyle umbellata* (dollar weed). The same endornaviruses were also detected in at least 10 individual plants of the same species. RT-PCR tests using endornavirus-degenerate primers were conducted with the dsRNAs of the six putative endornaviruses. Results of this investigation suggest that endornaviruses occur at a rate of approximately 5% in non-cultivated plant species and that like endornaviruses of cultivated plants, their vertically transmission rate is high.

#### **Development and validation of a quadruplex real-time RT-PCR assay for simultaneous detection of three *Citrus leprosis* viruses in plants**

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*Citrus leprosis virus* (CiLV) is the causal agent of Citrus leprosis - one of the most destructive diseases of citrus. CiLV is an RNA virus transmitted by *Brevipalpus* mite species. There are two major types of CiLV being described – one is the cytoplasmic type residing in the cytoplasm of infected cells and the other is the nuclear type primarily residing in the nucleus of infected cells. In the last decade CiLV spread swiftly from South America to Central America and recently has been detected in Mexico. The proximity and the potential threat to the multi-billion dollar US citrus industry have caused concerns in the United States where the disease has not been found since the 1960s. Molecular identification and early detection of each the cytoplasmic and nuclear types are essential to prevent CiLV from introduction and establishment in the US. In order to reduce testing time, cost and labor, we are developing and validating a quadruplex real-time RT-PCR assay for simultaneous detection of cytoplasmic CiLV-C, CiLV-C2, and nuclear CiLV-N with a Nad5 plant RNA internal control. From our repeated experiments, the results showed that sensitivity and specificity for the quadruplex RT-PCR assay were comparable to the level of monoplex RT-PCR detecting CiLV-C, CiLV-C2, and CiLV-N respectively. Further validation for the quadruplex RT-PCR assay including tests with additional field samples is in progress.

#### **A sociological assessment of *Potato virus Y* in western Washington: Barriers and bridges to adopting new management practices**

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Seed and commercial potatoes are grown in separate geographic areas of western Washington (WWA). In 2012, recombinant strains of *Potato virus Y* (PVY), PVY<sup>N<sup>TN</sup></sup> and PVY<sup>N<sup>WI</sup></sup>, were reported in WWA potato fields. The strains are largely asymptomatic on potato foliage and difficult to detect by visual field inspections and winter greenhouse grow-out tests. The problem led to tuber yield and quality losses of ~40% for commercial producers who purchased inaccurately certified seed. In 2015-16, most (N=6) of the seed potato growers in WA and approximately one-third (N=6) of the large-scale commercial potato growers in WWA were interviewed about the barriers and bridges to adopting updated PVY detection approaches such as ELISA and PCR-based laboratory tests, tropical winter field grow-outs, and PVY recognition trainings. The major barriers to adoption were logistical learning curves (67%), uncertainty that updated techniques would be advantageous (67%), and distrust of laboratory results (50%). The major bridges included premiums paid by customers for reliable PVY-free seed (75%), customer demand for more sensitive PVY testing methods (67%), and faster grow-out results (33%). The study results can guide researchers and extension educators who make PVY management recommendations that must be relevant to the needs and perceptions of growers. Further, they suggest that sociological studies of plant pathogens can provide a valuable tool for assisting in plant disease management.

#### **Rapid and sensitive detection of tomato chlorotic dwarf viroid in crude plant extracts using isothermal RT-recombinase polymerase amplification**

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Tomato chlorotic dwarf viroid (TCDVd) is a serious threat to tomato production worldwide. Infection leads to reduced plant vigor, small and deformed fruit, and yield loss. TCDVd is easily transmitted mechanically from plant to plant once introduced and through seed; seed treatments do not control transmission of disease. The most effective control is the use of certified viroid-free planting materials. We developed a molecular diagnostic assay utilizing reverse transcription-recombinase polymerase amplification (RT-RPA) at an isothermal constant temperature of 39°C and target-specific primers and probe for the rapid, sensitive, and specific detection of TCDVd in crude extracts of infected leaf and seed tissues. The performance of the AmplifyRP<sup>®</sup> Acceler8<sup>™</sup> RT-RPA diagnostic assay, utilizing a lateral flow strip contained within an amplicon detection chamber, was evaluated and the results were compared with a standard RT-PCR assay. The AmplifyRP<sup>®</sup> Acceler8<sup>™</sup> assay was specific for TCDVd and its sensitivity was comparable to conventional RT-PCR. The assay does not require extensive sample purification, specialized equipment, or technical expertise. It is easy to perform and can be used under both laboratory and field conditions.

#### **Distribution and vertical transmission of Southern tomato virus in tomato**

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*Southern tomato virus* (STV) is a recently identified virus that infects tomato cultivars worldwide. STV has a monopartite virus with a double-stranded RNA genome (3.5 kb) containing two overlapping open reading frames (ORFs) and is the type species of the genus *Amalgavirus* (family



*Amalgaviridae*). A reverse-transcription polymerase chain reaction (RT-PCR) using virus-specific primers was used to detect STV in 21 tomato cultivars. STV was detected in plants produced by a number of seed companies which indicates that the virus is widely distributed in the vegetable seed industry. STV was detected for the first time in four cultivars: 'Agrisret 761', 'Mexico Midget', 'Roma' and 'Sweet Hearts'. 'Sweet Hearts' tomato plants were evaluated for the presence of STV using RT-PCR at different developmental stages of tomato plants. STV could be detected in the 1 to 2 week-old germinated seedlings at a frequency similar to that of older plants from the same seed lot. The presence or absence of STV was consistent across all four developmental stages. Seed transmission studies of progeny produced from crosses of different combinations of parents that were STV-positive or negative, revealed that STV could be transmitted to the next generation by either ovule or pollen.

#### **Emergence of a novel white stripe disease pathogen *Stenotrophomonas maltophilia* strain Sia5 in rice fields of Punjab under the climate change scenario**

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Under severe fluctuations in temperature and rainfall patterns several previously unimportant pathogens are gaining importance especially in chief cash crops in many parts of the world. Recent surveys over the last three years in the rice Kallar belt districts of Punjab (Kala Shah Kaku, Hafizabad, Sheikhpura, Narawal, Shakargarh, and Sialkot) depicted the presence of a novel disease and its associated pathogen which is responsible for the appearance of white stripe symptoms on leaves that start from the leaf tip and extend along the midrib through the leaf lamina towards the base of the leaf sheath. Under severe infection leaf size got reduced, whole leaves got dried and panicles matured very late. Diseases incidence ranged from 25-30% inflicting 20-25% yield losses. Pathogen was isolated on nutrient agar that formed dark to pale yellow raised colonies with fluffy appearance and was reconfirmed through pathogenicity testing on susceptible rice variety. The most aggressive and most virulent isolate belonged to Sialkot region and was characterized as *Stenotrophomonas maltophilia* strain Sia5 through the BLAST searches and phylogenetic analysis of its PCR amplified 16srDNA sequence (GB: KT924441.1) and other biochemical characteristics. This is the first report on the occurrence of white stripe disease in rice from Pakistan.

#### **An invasive lethal phytoplasma disease threatening stone fruit production: Detection, epidemiology and management**

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Introduced into North Lebanon in the early 1990s, almond witches' broom (AlmWB) disease spread rapidly in Lebanon killing about 200,000 almond, peach and nectarine trees. The disease is associated with '*Candidatus* Phytoplasma phoenicium' that belongs to 16S rRNA subgroup IX-B. The major symptoms are proliferation, small chlorotic leaves, development of witches' broom followed by dieback and death of almond trees. So far, AlmWB has been reported in Lebanon and Iran. In Lebanon, AlmWB was considered of quarantine importance and official containment measures were initiated in major stone fruit production areas. Specific, sensitive and reliable PCR and real-time PCR detection methods were developed. Preliminary data revealed that a leafhopper and planthoppers are vectors of the disease and two weed species are suspected to act as alternative hosts. An integrated disease management approach is considered for highly infested regions. Grafting experiments are in progress and preliminary results look encouraging, a recovery phenomenon was observed. Finally, regional and international cooperation are highly recommended to continue studies on the epidemiology of this invasive disease and to adopt phytosanitary control measures and strategies in order to contain the further spread of the disease.

#### **Root and shoot susceptibility of peach/almond rootstock to pistachio bushy top syndrome isolates of *Rhodococcus* spp.**

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Approximately 30,000 acres of UCB-1 clonal pistachio rootstock planted in California from 2011-2014 were affected with pistachio bushy top syndrome (PBTS) caused by two phytopathogenic bacteria highly related to *Rhodococcus fascians* (*Rf*) and *Rhodococcus corynebacterioides* (*Rc*). Orchards with high disease incidence were removed and replanted to pistachio or almond. The susceptibility of almond rootstock to PBTS isolates is unknown. Objectives include determination of the susceptibility of peach/almond rootstock to PBTS isolates and the influence of root and shoot infections on symptom development and host colonization. Either roots or shoots of clonal 'Hansen 536' rootstock were dipped in a bacterial suspension containing both PBTS isolates. Uninoculated control plants were dipped in buffer solution. Plant growth metrics and shoot and root biomass were determined 8 weeks after inoculation, and leaves were sampled for endo- and epiphytic populations of *Rf* and *Rc*. Root-inoculated plants were stunted and exhibited increased node density in comparison to control plants. Foliar-inoculated plants remained asymptomatic, but foliar inoculation resulted in endophytic colonization of leaves. Root-inoculations resulted in aboveground plant colonization. The results demonstrate that 'Hansen 536' rootstock is susceptible to the PBTS isolates. The results indicate a need for field evaluation for disease in PBTS-affected orchards replanted to almond.

#### ***Xylella fastidiosa* isolates differ in the ability to undergo genetic recombination**

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*Xylella fastidiosa* (*Xf*) is a bacterium causing incurable diseases in economically important crops such as grapevines, citrus, and olives. Historically assumed to be restricted to the Americas, *Xf* diseases have been reported in both new locations and new crops in recent years. Inter-subspecific recombination (ISR) has been proposed to contribute to host shifts based on genetic diversity studies. Natural competence, as a mode of recombination, has been shown to occur in vitro in systems mimicking natural habitats. However, little is known about the recombination potential of *Xf* isolates, which are believed to be somewhat host specific. Therefore, in this study we compared the recombination frequencies of twelve *Xf* isolates belonging to two subspecies (*fastidiosa* and *multiplex*), using plasmids containing antibiotic markers flanked on either side by *Xf* homologous regions. The recombination frequency varied greatly ( $3.14 \times 10^{-2} - 2.3 \times 10^{-8}$ ) among isolates. Generally, subsp. *fastidiosa* isolates had higher recombination frequencies than *multiplex* isolates, and within a subspecies, the most virulent isolates had higher frequencies. When combinations of marker-tagged, heat-killed donor and live recipient cells from two subspecies were mixed, ISR occurred with a genomic region of ~10kb transferred from the donor to the recipient cell. This study demonstrates different recombination abilities, and therefore suggests different evolutionary potentials among *Xf* isolates.

#### **Soil survival potential of pistachio bushy top syndrome isolates of *Rhodococcus* spp.**

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Over 35,000 acres of pistachio planted from 2011-2014 in California were affected by pistachio bushy top syndrome (PBTS), a disease caused by bacteria related to *Rhodococcus corynebacterioides* (*Rc*) and *Rhodococcus fascians* (*Rf*). Because the survival potential of PBTS isolates in soil is unknown, growers are reluctant to replant pistachios in affected orchards. The goal of this work was to assess the longevity of *Rc* and *Rf* in field soils

exposed to environmental conditions and in potting medium under greenhouse conditions. An *in situ* field study was established in a PBTS orchard with each isolate embedded on wooden sticks and buried at various depths. Another study utilized excavated field soils incubated outdoors to investigate the influence of soil type and moisture on survival. In a greenhouse study, potting medium was infested with *Rf* in the presence and absence of dried pistachio plant material. After three months, populations of *Rf* in potting medium remained similar to initial populations, regardless of incorporation of plant tissue. After one month, *Rf* populations increased in moist, non-saline field soil, but *Rc* was almost eliminated. Both isolates were inhibited by salinity and drying, but depth did not influence survival. The results suggest saprophytic competence of *Rf*, and a consequent concern for persistence of *Rf* in affected orchards and nurseries and illustrate the need for vigilance in sanitation from the nursery to the field.

**Bacterial black spot of mango, *Mangifera indica*, caused by *Xanthomonas citri* pv *mangiferaeindicae*, is confirmed in the Western Hemisphere**

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During the summer of 2015, symptoms of what appeared to be bacterial black spot (BBS) were observed on mature fruits of mango, *Mangifera indica*, in the vicinity of Boynton Beach, FL. Star-shaped lesions, up to 2 cm in diameter, erupted from the surface of 'Keitt' and other cultivars, and oozed a sticky, clear exudate. From lesions, a cream-colored bacterium was recovered on YPGA and characterized with housekeeping genes used by Bui Thi Ngoc et al. (2010) (*efp* and *dnaK*). Amplicons from a representative isolate were sequenced (KU746336 and KU746337) and were 100% homologous with strains of *Xanthomonas citri* pv *mangiferaeindicae* (Xcm) in GenBank. ML analysis (Tamura-Nei model) with MEGA 6.06 placed the isolate among other strains of Xcm. In incubator studies (30°C day, 26°C night, 12h light), the mesophyll of leaves on potted 'Keitt' and 'Haden' plants were injected with a hypodermic needle and  $1 \times 10^5$  CFU mL<sup>-1</sup> of the isolate. After 7 days, black lesions developed on inoculated leaves, but not on leaves injected with sterile deionized H<sub>2</sub>O. Diagnostic amplicons for Xcm were generated for isolates recovered from the lesions, and the experiment was conducted twice. BBS is widespread in the Eastern Hemisphere, but this is the first report of the disease in the Western Hemisphere. Environmental conditions in southern Florida appear to be ideal for an increased importance of BBS in the region.

**Survey on pathogenic *Xanthomonas* recovered from different lettuce cultivars in Canada**

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Bacterial leaf spot, caused by *X. hortorum* pv. *vitians* (*Xhv*), is an important lettuce disease. In Canada, the main difficulty for disease control is the absence of registered chemicals. Thus, the most sustainable way to reduce yield losses is using resistant cultivars. Preliminary studies showed that different isolates did not share the same lettuce host range. Therefore, different *X. hortorum* pathotypes should be considered in a breeding program for resistance. The objective was to determine which pathotypes are present in Canada by sampling diseased lettuce plants in commercial production. In 2014 and 2015, 376 lettuce samples were collected in the main lettuce production area. *Xanthomonas* isolates were recovered from leaf lesions and characterized based on DNA sequences of four housekeeping genes (*dnaK*, *fyuA*, *gyrB* and *tpoD*). Concatenated DNA sequences of these genes for the isolates and reference strains were analyzed with Neighbor-Joining method using MEGA v6.04 to generate a phylogenetic tree. The 363 isolates formed two major clusters. Cluster I includes 17 strains representing 358 isolates and grouped with the *Xhv* reference strain BS3046. This Cluster can be separated into two sub-groups and preliminary evidence shows that they shared different lettuce host range. The Cluster II, a distinct group of *X. hortorum*, contains eight new strains, but the pathotype has to be determined. Further investigation is needed on the epidemiology of this unknown pathotype.

**Wheat blast is caused by multiple *Pyricularia* species, including *Pyricularia graminis-tritici* sp. nov.**

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Presently, *Pyricularia oryzae* pathotype *Oryza* (Po-O) is considered the widely distributed rice blast pathogen, whereas *P. oryzae* pathotype *Triticum* (Po-T) is the South American wheat blast pathogen. In this study, we investigated whether Po-O and Po-T were distinct at species level. We also described a new *Pyricularia* species causing blast on several Poaceae, including wheat, from Brazil. Bayesian phylogenetic analyses were conducted on 10 genes from an extensive sampling ( $N = 128$ ) of populations of *Pyricularia* adapted to rice, wheat and other Poaceae. Isolates were grouped into two major clusters (Clusters I and II,  $P=0.99$ ). Cluster I contained isolates from wheat and another Poaceae ( $P=0.98$ ). Cluster II was sub-divided in three clades ( $P>0.75$ ). Clades 1 and 2 grouped wheat-derived isolates, and Clade 3 contained rice-derived isolates. The Clusters I and II corresponded to two distinct species: *Pyricularia graminis-tritici* sp. nov. (Pgt), newly described here, and *Pyricularia oryzae* (Po). The clades of Po concurred with Po-T and Po-O, and were not distinct at species level. Among species, morphological differences were not recorded; however, a distinctive pathogenicity spectrum was observed. Pgt and Po-T were highly virulent on wheat, barley, signal grass, and oats. Po-O was highly virulent on rice, wheat, barley, and oats, but not on signal grass. We demonstrated that blast disease on wheat and other Poaceae from Brazil is caused by multiple *Pyricularia* species.

**Unraveling Silverleaf disease: Reversion of foliar symptoms and plants recovery**

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Silverleaf, caused by the Basidiomycete *Chondrostereum purpureum*, is an important disease in fruit trees which produce wood necrosis and leaves silverying. In apple has been increasing due to changes in management, reducing orchards viability. Plants deteriorate their physiological condition, being water uptake and chlorophyll content the most affected parameters. The latter alters photosynthesis, reducing fruit yield and quality. Field studies have shown the spontaneous reversion of foliar symptoms, thus, the objective was to compare the physiological and sanitary condition of Brookfield plants before and after reversion. The sanitary condition was determined assessing symptoms severity, isolating the fungus on PDA, amplifying the large intergenic spacer (IGS) rDNA region and measuring foliar endopoligalacturonase concentration using DAS-ELISA. Physiological condition was measured through xylem water potential, chlorophyll fluorescence, a, b and a + b chlorophyll content and stomatal conductance. Besides, yield and fruit quality parameters such as color and starch were assessed. Isolation, PCR and ELISA indicated that the fungus was present in symptomatic plants and after reversion. Symptomatic plants presented alterations in all physiological parameters ( $P<0,05\%$ ), reduction of yield (38%), maturation disorders and lack of color. However, after reversion of silverying, physiological condition, yield and fruit quality were restored.

**Fungi in the wood: The fungi associated with the ambrosia beetle, *Xylosandrus germanus* and its galleries in *Malus domestica***

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The ambrosia beetle, *Xylosandrus germanus*, is considered an emerging threat to Western New York apple orchards. There are concerns that fungal farms established by *X. germanus* within vascular tissue play a role in the collapse of young apple trees. To gain further insight into the identity and relationship between fungi associated with *X. germanus* and decline of *Malus x domestica*, fungal isolations were performed from *X. germanus* wood

galleries and adjacent vascular tissue, and beetles recovered from galleries. Fungal identity was confirmed by sequencing the 2 internal transcribed spacer regions and the 5.8S gene of ribosomal DNA. Five distinct fungal species were recovered from the exterior of 36 beetles, while only 2 fungal species were recovered from the beetles' interior. *Nectria haematococca* (an. *Fusarium solani*) was most frequently isolated from the exterior and interior of *X. germanus*, while *Ambrosiella xylebori*, another common food source of ambrosia beetles, was only isolated from the interior and exterior of one beetle. Interestingly, however, *A. xylebori* was predominantly recovered from infested wood tissue. Our results suggest that *X. germanus* can establish fungal gardens of *N. haematococca* and *A. xylebori* within vascular tissue of apple trees, however it is unclear if tree decline is likely due to direct tunneling or colonization of *A. xylebori*. Additional investigations are ongoing to understand the implications of *A. xylebori* colonization of apple.

#### **High diversity in the population of an emerging blackberry pathogen, *Fusarium oxysporum***

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A wilt disease of blackberry, caused by the soilborne fungus *Fusarium oxysporum*, has become a more widespread problem in California over the past two years. This emerging pathogen is severely reducing fruit production in affected fields. To improve our understanding of the structure of pathogen population, isolates collected from symptomatic blackberry plants in California and Mexico were evaluated for morphology, pathogenicity and vegetative compatibility. The translation elongation factor 1 alpha (TEF1- $\alpha$ ), the intergenic spacer (IGS) and the  $\beta$ -tubulin regions were also sequenced. The combination of TEF1- $\alpha$ ,  $\beta$ -tubulin and IGS sequences identified clusters of isolates that corresponded to vegetative compatibility groups (VCGs). A high number of distinct populations were founded both in California and Mexico. However, one VCG included isolates from both countries. No relation was found between VCG and cultivar or geographic origin. Diversity in the pathogen population suggests that the capacity to cause disease on blackberry is not a recent development.

#### **Mucor Rot – An Emerging Postharvest Disease of Mandarin Fruit Caused by *Mucor piriformis* and other *Mucor* spp. in California**

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In recent years, an emerging, undescribed postharvest disease was observed on mandarin fruit after extended storage in California. We collected decayed mandarin fruit from three citrus packinghouses in the Central Valley of California in 2015 and identified this disease as Mucor rot caused by *Mucor* spp. Mucor rot occurred in 11 of the 15 grower lots sampled, and the incidence of the disease in the total decayed fruit varied among affected grower lots, ranging from 3.3 to 93.1% with an average of 49.2%. In total, 197 isolates of *Mucor* spp. were obtained from decayed mandarin fruit and identified based on ITS sequence and morphological characteristics. Of the 197 isolates, 92.4%, 3.6%, 2.0%, 1.5%, and 0.5% were identified as *M. piriformis*, *M. circinelloides*, *M. racemosus*, *M. hiemalis*, and *M.ucedo*, respectively. Pathogenicity tests on mandarin fruit showed that *M. piriformis* was the most virulent among all tested species. Our results indicated that Mucor rot of mandarins in California is caused by *Mucor* species consisting of *M. piriformis*, *M. circinelloides*, *M. racemosus*, *M. hiemalis*, and *M.ucedo*, with *M. piriformis* being the most prevalent species. Previously, *M. racemosus* was reported on citrus. This is the first report of Mucor rot on citrus caused by *M. piriformis*, *M. circinelloides*, *M. hiemalis*, and *M.ucedo*.

#### **Botrytis californica, a new *Botrytis* species causing gray mold in blueberries and table grapes in California**

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The *Botrytis cinerea* species complex comprises two cryptic species, originally referred as Group I and Group II based on *Bc-hch* gene RFLP haplotyping. Group I was described as a new cryptic species *B. pseudocinerea*. During a survey of *Botrytis* spp. causing gray mold in blueberries and table grapes in the Central Valley of California, six isolates, three from blueberries and three from table grapes, were placed in Group I but showed a distinct morphological character with conidiophores significantly longer than those of *B. cinerea* and *B. pseudocinerea*. We compared these isolates with *B. cinerea* and *B. pseudocinerea* by examining morphological and physiological characters, sensitivity to fenhexamid, and phylogenetic analysis inferred from sequences of three nuclear genes. Phylogenetic analysis using the three partial gene sequences encoding glyceraldehyde-3-phosphate dehydrogenase (G3PDH), heat-shock protein 60 (HSP60), and DNA-dependent RNA polymerase subunit II (RPB2), supported the proposal of a new *Botrytis* species, *B. californica*, which is genetically closely related to *B. cinerea*, *B. pseudocinerea*, and *B. sinoviticola*, all known as causal agents of gray mold of grapes. *B. californica* caused decay on blueberry and table grape fruit inoculated with the fungus. This study suggests that *B. californica* is a cryptic species sympatric with *B. cinerea* on blueberries and table grapes in California.

#### **Multiple *Pestalotiopsis* and *Neopestalotiopsis* species cause flower blight of macadamia in Australia**

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Incidence of 'dry flower' the disease, has been increasing and now occurs in different production regions in Queensland and New South Wales, Australia. Although yield losses range between 10% and 30%, total crop failures have occurred in certain cultivars. Therefore, this study examined the etiology of the disease in Australia. Results from a survey of over 240 macadamia racemes in different orchards in Australia revealed that all the four developmental stages of macadamia racemes including the florets and the rachis were affected. Several fungi genera including *Alternaria*, *Epicoccum*, *Fusarium*, *Botrytis*, *Cladosporium*, *Penicillium*, *Aspergillus* and *Diaporthe* were obtained from tissue samples, however, their occurrence was irregular between samples and significantly ( $P < 0.05$ ) less than *Pestalotiopsis*-like isolates, which constituted 41% of the total isolates. Based on the concatenated partial sequence of three gene loci (Internal transcribed spacer,  $\beta$ -tubulin and translation elongation factor 1-alpha) the phylogenetic analysis of 32 *Pestalotiopsis*-like isolates showed they formed two novel taxa in the *Neopestalotiopsis* and *Pestalotiopsis* genera. The grouping of the isolates was regardless of location or plant part. Koch's postulates were confirmed with the different *Neopestalotiopsis* and *Pestalotiopsis* species and this is the first report of these two genera as causal agents of raceme blight of macadamia.

#### **Cytospora leucostoma is the most virulent and prevalent causal agent of Cytospora Canker on peaches on the Western Slope of Colorado**

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Cytospora canker on peaches (*Prunus persica* (L.) Batsch) is known to be caused by three species, *Cytospora cinctum*, *C. leucostoma*, and *C. paraleucostoma* within growing areas of the United States. In Colorado, recent surveys show that over 90% of the trees on the Western Slope are infected with Cytospora canker, causing significant economic impacts to peach growers. Understanding how these species differ phenotypically and in disease cycle and progression is important for disease management. The objectives of this study were to identify *Cytospora* isolates collected from high and low elevation sites, examine population structure and recombination, and assess each species/population for phenotypic characters such as virulence, temperature growth and culture morphology. The ITS region was sequenced to identify 106 isolates. Both *C. leucostoma* and *C. paraleucostoma* were recovered, although *Cytospora leucostoma* was the most prevalent. Additional sequencing of 4 genetic loci (ca. 1950 bps), suggested that *C. leucostoma* and *C. paraleucostoma* recombine and that well-supported clades do not exist for 3 of the 5 loci. Phenotypic characterization indicates overlapping

variation in temperature, growth, culture morphology, and virulence. These data suggest that isolates are genetically distinct populations of *C. leucostoma* rather than cryptic species.

#### **New *Botrytis* species affecting strawberry on the east coast**

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We detected a new species of *Botrytis* infecting strawberries in the United States. While monitoring fungicide resistance of 459 *Botrytis* isolates collected from South Carolina (211), Maryland (123), North Carolina (101), Virginia (17), and Ohio (7), we observed isolates with phenotypes different from typical *Botrytis cinerea*. These isolates produced fluffy, white mycelium and sporulated poorly on potato dextrose agar, minimal medium, strawberries, apples, and tomatoes. These isolates were found in 11 different locations across South Carolina (20), and North Carolina (4). The G3PDH gene was sequenced for these isolates and nucleotide BLAST search revealed that they formed a cluster separate from *Botrytis* species previously described. Further sequencing of RPB2, Hsp60, NEP1, and NEP2 genes for three representative isolates revealed that these isolates were identical to isolates of *Botrytis fragariae*, a species recently discovered infecting strawberry in Germany, but never before observed in the United States. In order to determine the distribution and prevalence of this species in the eastern United States, primers were developed that distinguish *Botrytis cinerea* and *Botrytis fragariae*. Preliminary data suggest that *Botrytis fragariae* isolates are more likely to have reduced sensitivity to the new fungicide polyoxin D. The discovery of a new species of *Botrytis* causing gray mold of strawberry may require adjustments in gray mold management strategies.

#### **Laurel wilt, caused by *Raffaelea lauricola*, is detected for the first time outside the southeastern USA**

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Agriculture is an important industry in Myanmar (aka Burma), accounting for 60% of the GDP and employing 65% of the work force. In October 2014, a survey for diseases and pests of an emerging fruit crop in the country, avocado (*Persea americana*), was conducted in Southern Shan State. Around Tuanggyi, monocultures of up to 20 hectares have been established, whereas avocado is an over-story tree where coffee is grown in the Ywangan area. Symptoms of laurel wilt, including sapwood discoloration, leaf necrosis, defoliation, and tree mortality were observed during the survey in both areas. In Myanmar, isolates that resembled the laurel wilt causal agent, *Raffaelea lauricola*, were recovered from symptomatic sapwood on ½ strength potato dextrose agar + streptomycin sulfate. In the USA, *R. lauricola*-specific amplicons were generated for isolates from eight different trees with PCR primers for two taxon-specific microsatellite loci. In a quarantine greenhouse in Gainesville, FL, three of the isolates caused laurel wilt in each of two tests on avocado and swamp bay (*P. palustris*), with disease severities similar those caused by an isolate of *R. lauricola* from the USA. Although *R. lauricola* was probably introduced from Asia to the southeastern USA with its ambrosia beetle symbiont, *Xyleborus glabratus*, it had previously been reported only in the southeastern USA. This is the first report of laurel wilt in the Asian homeland of *R. lauricola* and *X. glabratus*.

#### **Pathogenicity tests confirm *Macrophomina phaseolina* as an aggressive fungal pathogen of flowering dogwood (*Cornus florida* L.)**

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Pathogenicity tests where seedlings of flowering dogwood (*C. florida* L.) were challenged with extracted spores of *Macrophomina phaseolina* from 10 day-old cultures, while control were mocked inoculated with plain malt extract agar in sterile deionized water, demonstrated the capability of the pathogen to cause severe root rot in *C. florida*. Roots of juvenile seedlings vernalized in sterilized vermiculite; then grown on large water agar petri plates overlaid with sterile, moisten-filter paper, after being inoculated with the pathogen, showed high percentage of necrotic tissue in the first 10 mm of root tips. Concurrently, juvenile seedlings grown in large containers after similar treatment exhibited presence of fungal structures in roots from challenged plants that were not observed in the control. On 35 day-young dogwood seedlings grown in sterile soil under greenhouse conditions and inoculated on wounded stems, fungus-treated plants produced decay and large canker-like lesions that were not on the control. The extent of fungal growth in millimeter (mm) as the index to evaluate the host's response(s) showed marked difference between treatments and control. The level of aggressiveness of the pathogen was determined by measuring the duration and number of dead tissues or plants per given time, and cell death confirmed the amount of infection inside the roots.

#### **Powdery mildews on native plants in Utah**

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Many people want to grow more native plants in their yards. However, there is little information on plant diseases of native plants of the western United States. Powdery mildew is very common in Utah on many cultivated plants. The purpose of this project was to determine the presence of powdery mildew on native plants and identify the powdery mildew species using morphological and molecular techniques. Plants from the families Asteraceae, Geraniaceae and Polemoniaceae with powdery mildew were collected from a high elevation mountain valley (2,750 meters) in Northern Utah in fall 2015. Chasmothecia and conidia were collected from the plant tissue for morphological identification using a compound microscope. Identification to genus and/or species was confirmed using polymerase chain reaction (PCR) amplifying the internal transcribed spacer (ITS) region and DNA sequencing. The resulting sequences were compared in a BLAST search to powdery mildew sequences in the NCBI GenBank database. Several species of powdery mildew including *Podosphaera* sp. on sticky purple geranium (*Geranium viscosissimum*) which to our knowledge is the first report of powdery mildew on this species in the United States and *Golovinomyces magnicellulatus* on *Polemonium* sp., the first report of this powdery mildew in Utah.

#### **Diaporthe and spruce decline: Incidence, pathogenicity, and population genetics**

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Spruce decline, characterized by needle loss and branch dieback, is found throughout Michigan. A survey of fungi from cankers in the Lower Peninsula of Michigan found *Diaporthe* (formerly *Phomopsis*) to be the most common, disease-causing fungus. Sequencing *Diaporthe* isolates using ITS1 and ITS4 established 5 different groups. Pathogenicity studies were performed on these groups by inserting mycelial agar plugs containing a representative isolate of each group into a small wound, sealing the wound with Parafilm, and measuring the canker area 9 weeks post-inoculation. Groups 4 and 5 were the most virulent, while group 3 was the least virulent group. Groups 1 and 2 were intermediate on the virulence spectrum. Colorado blue spruce (*Picea pungens*) was the most susceptible species, and 9 different seed sources of *P. pungens* did not differ in susceptibility. Other tree taxa tested, in order from most to least susceptible, were Norway (*P. abies*), white (*P. glauca*), Black Hills (*P. glauca* var. *densata*), Serbian (*P. omorika*), and Meyer (*P. meyeri*) spruce. Group 1 was more common in the eastern Michigan, and fell into a *Diaporthe eres* clade. Group 3 was more common in the central region, and also fell into a *D. eres* clade. Group 2 was most common in the central region, and was unresolved taxonomically. Groups 4 and 5 were common throughout the Lower Peninsula and were also unresolved. Future work includes sequencing more gene regions to resolve *Diaporthe* groups.

### **New canker disease of Incense-cedar in Oregon caused by *Phaeobotryon cupressi***

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Incense-cedar (*Calocedrus decurrens*) is a tree native to Oregon and California. Since the early 2000's, a canker disease has been observed with increasing frequency on ornamental and windbreak trees planted in the Willamette Valley of Oregon. Symptoms appear as dead, flagging, small-diameter (<1 cm) branches that are scattered throughout the crown. *Phaeobotryon cupressi* was consistently isolated from symptomatic trees located along the length of the Willamette Valley. Identification was based on morphology and on sequences from the internal transcribed spacer (ITS) and the translation elongation factor 1- $\alpha$  (EF1- $\alpha$ ) region. Six isolates were tested for pathogenicity on potted 0.6-1 m tall incense-cedar saplings. Approximately 1 to 1.5 months after inoculation, inoculated branches began to turn brown and die, while control branches that received no inoculum remained healthy. *P. cupressi* was described as a new species in 2009, and was originally found causing cankers on Italian cypress (*Cupressus sempervirens*) in Iran. The pathogen was also detected once in the U.S. from *Juniperus squamosus* in Kansas. It is unknown whether *P. cupressi* affects native stands of incense-cedar, where similar symptoms have been observed, but studies are underway to evaluate the extent of this disease in Oregon.

### **The impact of laurel wilt caused by *Raffaelea lauricola* on clonal populations of pondberry (*Lindera melissifolia*)**

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Pondberry is a rhizomatous, clonally reproducing, woody shrub species that is federally endangered. Like other members of the Lauraceae that occur in the United States (US), pondberry is highly susceptible to laurel wilt, a disease caused by *Raffaelea lauricola* and vectored by the redbay ambrosia beetle (*Xyleborus glabratus*). A study was initiated in 2012 to assess the potential impact of the disease on clonal populations of pondberry. Individual pondberry plants were planted in nursery beds near Athens, Georgia and grown for approximately 3 years, and during this time individual plants produced many ramets. The original plant in two populations were inoculated with *R. lauricola* in July, 2015 and plants were monitored regularly. After 12 wk, 55 ramets died in one population at distances up to 172 cm from the original inoculated plant, and in the other population 22 ramets died at distances up to 337 cm. *R. lauricola* was reisolated from 84% of the wilted ramets. The study confirms that pondberry is highly susceptible to laurel wilt and that the disease can spread rapidly through rhizomes of infected plants. Although pondberry is a small shrub that is not often attacked by *X. glabratus*, when infections occur they could be detrimental to populations. Additional studies are being conducted to examine the feasibility of using trenching around infected plants to prevent disease transmission, and the use of chemicals to protect plants from infection.

### **The laurel wilt story: Introduction and impact of an exotic vector (*Xyleborus glabratus*) and pathogen (*Raffaelea lauricola*)**

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Laurel wilt is a lethal vascular disease that affects plants in the Lauraceae family. Since the exotic vector (*Xyleborus glabratus*) and its pathogenic fungal symbiont (*Raffaelea lauricola*) were introduced to the USA in 2002, laurel wilt has killed redbay (*Persea borbonia*) and related trees in southern forests. Many questions remain about the disease in the USA, including the number of introduction events, the genetic diversity of the vector and pathogen, and how many host trees have died. To infer the number of introduction events and assess genetic variability, Amplified Fragment Length Polymorphism (AFLP) markers were used to screen a collection of *R. lauricola* isolates, and a portion of the cytochrome oxidase I (COI) gene was sequenced and compared for *X. glabratus* samples. Results suggest that *R. lauricola* and *X. glabratus* are both clonal organisms in the USA that were likely introduced in a single event. Forest Inventory and Analysis (FIA) data were used to obtain pre- and post-epidemic redbay population levels, which were then used to calculate redbay mortality due to laurel wilt. The FIA data suggest that laurel wilt has spread over 250,000 km<sup>2</sup> and killed nearly half a billion redbay trees. These data highlight the devastating impact that invasive organisms can have on native forests.

### **Susceptibility of eight untested species of Cucurbitaceae to gummy stem blight under field conditions**

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To date, 23 species of 12 genera in the Cucurbitaceae are known to be affected by the cucurbit disease gummy stem blight (GSB), caused by the fungus *Stagonosporopsis* spp. (*Didymella bryoniae*). Eight different species representing seven genera of Cucurbitaceae were selected for taxonomic and geographic diversity. Muskmelon (*Cucumis melo* 'Athena'), which is highly susceptible, was included as a control. A field trial was set up as a randomized complete block design with four replications and seven inoculated plants per replication to assess the susceptibility of these species. The severity of the foliar blight phase and the incidence of the crown canker phase of GSB were rated. *Ecballium elaterium* showed the highest severity to foliar blight, followed by *Apodanthera sagittifolia* and *Kedrostis leloja*. These species were more susceptible than the control. A group of five species, *Acanthosicyos horridus*, *Cucurbita digitata*, *Cucurbita ecuadorensis*, *Cucumis zambianus* and *Melothria scabra*, were as susceptible to foliar blight as the control. The highest incidence of cankers was observed on crowns of *K. leloja* and 'Athena' muskmelon, followed by *C. zambianus*, *A. sagittifolia* and *E. elaterium* in decreasing order. The pathogen was recovered from crowns of all species. This is the first report of susceptibility of these eight cucurbit species to GSB.

### ***Raffaelea arxii* may be the primary symbiont of *Xyleborus affinis***

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Symbioses between fungi and ambrosia beetles are poorly understood. Most of the ambrosia beetles that have been studied have a primary fungal symbiont that predominates in a species' mycangium and natal gallery, where it is cultivated as a food source. However, there is increasing evidence that species of fungal symbionts can be laterally transferred to other beetle species (are not uniquely associated with a single species). For example, the primary symbiont of *Xyleborus glabratus*, *Raffaelea lauricola* (cause of laurel wilt), has been found in at least nine other species of ambrosia beetle. Symbiont composition was examined in several of the latter species. Mandibular mycangia of one of the species, *X. affinis* reared from laurel wilt-affected logs of avocado (*Persea americana*), were assayed for fungal communities. Partial sequences of LSU of rDNA regions identified high concentrations of phenotypically variable populations of *R. arxii* in 95%, whereas *Raffaelea* sp., *R. lauricola*, and *Ambrosiozyma platypodis* were recovered from, respectively, 45, 20 and 15% of 20 assayed individuals. Although *R. arxii* was first reported in South Africa as the primary symbiont of *X. torquatus* (syn. *X. volvulus*), the abundance and persistent association between *R. arxii* and *X. affinis* suggests that *R. arxii* may also be the primary symbiont of *X. affinis*. Primary and secondary symbionts of *X. affinis*, *X. volvulus* and other potential vectors of *R. lauricola* will be discussed.

### **Web blight of spinach in the desert southwest caused by *Pythium aphanidermatum***

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A unique foliar disease of spinach caused by *P. aphanidermatum* was observed in spinach in the Yuma, AZ/Imperial Valley, CA spinach production areas in October of 2015. The foliar symptoms of the disease included water soaked foliage, rapid collapse of young plants, with evidence of white cottony mycelium. The disease was associated with hot and wet conditions under high density (>3.0 M seed/A) plantings. Pure cultures were isolated from symptomatic spinach leaves and isolates were sequenced using ITS1/ITS4 primers. BLAST searches showed 100% homology with ITS sequences of *P. aphanidermatum* in GenBank. The objective of this research was to determine the effect of the *P. aphanidermatum* recovered on damping-off and foliar web blight on a broad range of hosts. Pathogenicity was tested in the greenhouse on spinach, cotton, soybean, pepper, tomato, cucumber, melon, squash, lettuce, corn, wheat, and rice, by either infesting the soil or doing foliar inoculations with colonized plugs of PDA. *P. aphanidermatum* caused severe damping off of all dicots tested, but only caused some seedling size reduction in corn and wheat. Web blight symptoms were severe with spinach, and all other dicot hosts. No web blight symptoms were observed on lettuce, corn, wheat, or rice. Metalaxyl provided complete protection for damping off of spinach and protected spinach plants for 10-20 days for U.S. treatment rates or 20-30 days after planting for E.U treatment rates, respectively.

### **Stone fruit survey efforts in Texas monitoring for Plum Pox Virus, European Stone Fruit Yellows & Light Brown Apple Moth: 2015-2016**

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The Texas Plant Disease Diagnostic Lab continues participation in the National Stone Fruit Survey in FY15, monitoring for pathogens of concern for the stone fruit industry in Texas. The Texas A&M stone fruit breeding plots and four (4) commercial peach orchards in 3 counties were tested for the presence of Plum Pox Virus (PPV) and European Stone Fruit Yellows (ESFY) phytoplasma in 2015. A total of 667 foliar peach and plum samples were tested for PPV. For virus detection, each sample was tested by ELISA using Agdia ELISA kits and following USDA APHIS PPQ and National Plant Diagnostic Network protocols. All 667 samples tested negative for all five strains of PPV. ESFY was not observed or detected in any of the surveyed peach orchards. This survey effort will continue through 2016, focusing on PPV, ESFY and the Light Brown Apple Moth (LBAM). This survey is monitor for possible introduction of these pests and allows for early detection.

### **Red rust of bromeliads caused by a parasitic alga in Florida**

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*Cephaleuros* is a genus of plant parasitic thalloid green algae containing at least two species *C. virescens* and *C. parasiticus* that are reported to cause disease on ornamental plants. Florida has 165 plant species belonging to 53 families that have been reported as hosts of *Cephaleuros*. Recent outbreaks of a “red rust” disease affecting bromeliads in production nurseries and landscapes led to an investigation of the causal agent, which was determined to be a *Cephaleuros* species. Morphological characteristics from *Cephaleuros* isolates recovered from *Neoregelia* hybrids match with isolates of *C. parasiticus* collected from leaves of *Psidium guajava* (guava). Molecular analyses were performed from 18S ribosomal RNA and ribulose-1,5-bisphosphate carboxylase/oxygenase large subunit (rbcL) genes. Pathogenicity tests were conducted using *Cephaleuros* isolates collected from leaves of *Neoregelia* hybrids and isolates of *C. parasiticus* from guava. Both produced symptoms consistent with those originally described as “red rust” on *Neoregelia* hybrids and the causal agent of red rust on *Neoregelia* hybrids provisionally will be *Cephaleuros parasiticus*.

### **First detection of the stubby root nematode *Paratrichodorus allius* on potato in North Dakota and on sugarbeet in Minnesota**

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Stubby root nematodes (SRN) are migratory ectoparasites that feed on roots, transmit tobnaviruses and cause significant crop loss. In 2015, 49 soil samples were collected from a potato field in Sargent County, ND of which seven contained SRN with population densities ranging from 135 to 300/kg soil. Subsequently, 13 soil samples were collected from a sugarbeet field in Clay County, MN and six of them had SRN from 200 to 1,000/kg soil. Individual SRN were examined morphologically and molecularly for species identification. The specimens from both potato and sugarbeet fields were identified as *Paratrichodorus allius*. Observations of female morphological characteristics critical for identification included rod like/oval and well separated vaginal sclerotization, onchiostyle 42-45µm, absence of lateral body pores, and small ventral pharyngeal overlap. DNA was extracted from single nematodes. D2/D3 region of 28S rRNA, partial 18S rRNA and ITS1 rDNA were amplified and PCR products were cloned and sequenced. The consensus sequence from each region was deposited into GenBank and compared with known sequences. The sequence information supported the identity as *P. allius*. *P. allius* is known to be the prevalent vector of *Tobacco rattle virus* causing potato corky ringspot disease. Until now, problems with infestations of SRN on sugarbeet have been confined to parts of Europe, California and Idaho. This represents the first occurrence of *P. allius* in North Dakota and Minnesota.

### **Phytophthora nicotianae causing foliar blight of *Codiaeum variegatum* and *Zamioculcas zamiifolia* in Florida**

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*Phytophthora* is an oomycete plant pathogen, commonly known as a ‘water mold’ that attacks all parts of the host plant, causing root, crown and stem rot and foliar blight. *Phytophthora* diseases affect numerous ornamentals in nurseries, garden centers and landscapes generally during the hot and humid rainy season in south Florida. *Phytophthora nicotianae* was isolated for the first time from diseased *Codiaeum variegatum* (‘variegated’ croton) and *Zamioculcas zamiifolia* (‘ZZ’ plant) plants. Initially the leaves appeared water soaked and eventually turned dark brown to black in both plants. Morphological characteristics, molecular analysis of the ITS region, and pathogenicity test revealed that *P. nicotianae* was the causal agent. Tropical foliage plants typically account for the greatest percentage of sales within the ornamental industry and we anticipate these diseases will have a substantial impact on tropical foliage in south Florida. Local tropical foliage producers lost 10% and 35% of croton and ZZ plant production, respectively. Therefore, it is important to enlist the use of effective, registered pesticides to provide preventative control of *Phytophthora* blight during the periods where consistent outbreaks occur.

### **Characterization of viral particles formed by *Grapevine vein clearing virus* (GVCV)**

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*Grapevine vein clearing virus* (GVCV) is closely associated with a grapevine vein clearing syndrome observed in Missouri and surrounding states. The virus was initially characterized through deep sequencing of small RNAs isolated from symptomatic grape tissue. Three overlapping fragments of GVCV genomes were cloned and sequenced, from which the GVCV genome was assembled. The nucleotide sequence revealed that GVCV belonged to the *Badnavirus* genus. However, GVCV virions have never been observed under electron microscope. A GVCV infectious clone has been constructed in order to characterize GVCV virions and to complete Koch’s postulates. The GVCV infectious clone was infiltrated into *Nicotiana benthamiana* young

leaves, and leaves were collected at 7 dpi, followed by a virion purification procedure involving ultracentrifugation through sucrose gradients. In contrast to the expected bacilliform virions, a flexuous rod structure was observed that had a 15nm diameter and varied in length from 83 to 930 nm. The same virion purification procedure was applied to GVCV-infected grape leaves showing typical vein clearing symptoms and two types of virion particles were observed from a single virion prep: a flexuous rod 15nm in width and up to 8,025 nm in length, as well as the typical bacilliform structure. Antibodies targeting the GVCV coat protein were designed from the GVCV coding sequence and will be applied to test the nature of these two potential GVCV particles.

#### **Survey for viruses of grapevine (*Vitis vinifera* L.) in coastal vineyards of Croatia**

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Viticulture in Croatia has a very long tradition representing an important branch of the national economy. Approximately 70 grapevine (*Vitis vinifera* L.) cultivars are considered to be autochthonous and are mostly grown in Croatian coastal region. Virus diseases of grapevines generally have a worldwide distribution and some have significant impact on vineyard longevity as well as quality and quantity of the fruit. This survey was initiated due to the limited data about the presence of economically most important viruses in Croatia. A survey was done on 10 autochthonous grapevine cultivars on a total of 48 vines from 22 vineyards. Extraction of total RNA was done from leaf petioles collected in August 2015 and vines were tested on the presence of 32 viruses by RT-qPCR. The most common virus found was GLRaV-3 (91,7%), followed by GVA and GRSPaV (81,3%), GFkV (68,8%), ArMV and GLRaV-1 (33,3%), GVB (10,4%), GPGV (8,3%) and GLRaV-2 (4,2%). Six viruses were detected for the first time in Croatia: GRVfV (14,6%), GRGV (12,5%), viruses from GLRaV-4 group, GVD and GVF (6,3%) and GSyV-1 (2,1%). Mixed infections were also common in individual grapevines. The survey indicates a poor sanitary status of autochthonous grapevine cultivars, demonstrating the necessity to extend work on sanitary selection programs.

#### **Blueberry green mosaic symptoms are associated with the presence of a new vitivirus**

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A virus was discovered in blueberry plants showing green mosaic symptoms, distinct symptomology from that associated with mosaic disease and was provisionally named as Blueberry green mosaic virus (BGMV). The genome was amplified using overlapping PCRs and has the typical organization of members of the genus *Vitivirus*, family *Betaflexiviridae* including five open reading frames (ORFs) and a 3' polyadenylated terminus. The ORFs code for the viral replicase, a 16 kDa protein of unknown function, the movement, coat and nucleic acid binding proteins, the latter presumably involved in RNA interference. Pairwise comparisons of the polymerase conserved domains of BGMV and established members of the genus showed amino acid identity ranging from 57-61% whereas phylogenetic analysis placed the virus in a distinct clade within the genus. The virus has been found in all plants showing green mosaic symptoms and its distribution and role in symptomology are currently being evaluated.

#### **Genome sequence analysis of a legume-infecting *Tomato chlorotic spot virus* isolate**

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*Tomato chlorotic spot virus* (TCSV, genus *Tospovirus*, family *Bunyaviridae*) has emerged as a significant constraint to economically important horticultural crops in Florida and the Caribbean region. In this study, we have determined the full genome sequence of a TCSV isolate from peanut (*Arachis hypogaea*) collected in Haiti. Total RNA isolated from virus-infected leaves was used to generate sequences of the S-, M-, and L-RNA genomic segments by a combination of conventional Sanger sequencing and next-generation sequencing (NGS). The consensus sequences generated from Sanger sequencing were compared against those derived from *de novo* assembly of the filtered NGS reads to confirm the quality and accuracy of the complete genome sequences of the three RNA segments. The 8,873 nucleotide (nt) L-RNA shared 98% nt identity with the L-RNA sequence of a TCSV isolate from Brazil. The 4,847 nt M-RNA shared 99% nt identity with the M-RNA sequence of a *Groundnut ringspot virus* and TCSV reassortant from Florida. The 3,310 nt S-RNA shared 32.5 to 73.8% nt identity with S-RNA sequences of other tospoviruses. Phylogenetic analysis of proteins encoded by individual TCSV genomic RNA segments with corresponding proteins encoded by other tospoviruses is in progress. This study demonstrates advantages of NGS methods for high throughput, insightful analysis of genome segment sequences of a multi-partite negative-stranded RNA virus directly from field infected tissue with no viral enrichment.

#### **Viral metagenomics of *Caladium x hortulanum* (Araceae) in Florida**

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Viral metagenomics and high-throughput sequencing were used to identify novel viruses present caladiums (*Caladium x hortulanum*, Araceae), collected from Lake Placid, Florida in 2013. Caladium cultivars showing viral-like symptoms were sequenced, reads were assembled and contigs were analyzed using BLASTX showing homology with viral species in the *Potyviridae*, *Rhabdoviridae* and *Caulimoviridae*. The bioinformatic results were validated by reverse transcription (RT)-PCR for RNA viruses and PCR for DNA viruses. Only two potyvirus species, *Dasheen mosaic virus* (DsMV), and *Konjac mosaic virus* (KoMV) were able to be confirmed by RT-PCR amplification and sequencing of the coat protein region (CP). DsMV was previously reported from caladium in Florida, however this is the first report of KoMV in Florida as well as North America. In order to explore the abundance and diversity of these viruses, 315 plants from 15 caladium cultivars were screened using RT-PCR with CP primers. DsMV was present in 69.5% of tested plants and in all cultivars. KoMV was present in 11% of tested plants, and in eight out of 15 cultivars. Sixty-one and thirteen CP sequences of DsMV and KoMV were sequenced, respectively. Pairwise comparison analysis indicated sequence similarity of 87.4% and 96.5% among isolates of DsMV and KoMV, respectively. The low diversity, abundance and phylogenetic analysis are consistent with a single introduction event for both DsMV and KoMV.

#### **Sequence variation between isolates of *Plantago asiatica* mosaic virus from lily and other hosts**

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*Plantago asiatica* mosaic virus (PIAMV) was reported from *Plantago asiatica* in the Russian Far East, and Nandina mosaic virus was reported from heavenly bamboo (*Nandina domestica*) in California, both in the 1970's. Nandina mosaic virus has not been reported to naturally infect any other hosts and was recognized as a strain of PIAMV (PIAMV-NMV) only when the full sequence was determined. In contrast, PIAMV was reported to affect lilies (*Lilium* spp.) and primrose (*Primula seiboldii*) in Japan, and in lilies in the Netherlands, Italy, Hungary, Spain, the UK, Taiwan, Chile, and the USA. Cut-flower Oriental and Asiatic hybrid lilies suffer significant losses of foliar and flower quality, or are even rendered unmarketable by PIAMV infection, showing foliar mosaic and necrosis, and streaking or distortion of the flowers. We have sequenced the coat protein (CP) gene and 3' non-coding region of PIAMV isolates from multiple imported lily cultivars, and compared these sequences to isolates from other countries and hosts, including PIAMV-NMV. Sequences from all imported lilies fall within the same clade as PIAMV isolates from Europe; lily and primula isolates from Japan fall into another clade; whereas PIAMV-type (*P. asiatica*) and PIAMV-NMV form monophyletic clades. PIAMV-type and PIAMV-NMV share multiple CP

amino acids differentiating them from other isolates; other residues are uniquely conserved within the 'European' clade, or within the 'Japanese' (lily and primrose) clade.

#### **Detection and characterization of a novel potyvirus, *Crinum mosaic virus*, in ornamental *Crinum***

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Ornamental flower bulbs (including true bulbs, bulbils, corms, tubers and rhizomes) are increasingly important floriculture crops. *Crinum* is a large genus of herbaceous perennial flowering bulbs in the family Amaryllidaceae and most of the species are grown in the summer and have large, showy, fragrant flowers. Only two viruses have been reported to infect cultivated *Crinum*, both potyviruses - *Nerine yellow stripe virus* (NeYSV; in India) and *Crinum mosaic virus* (CriMV; in Australia, FL and HI). CriMV has not been characterized and infection has only been noted by the presence of potyvirus particles or characteristic potyviral inclusions. Leaf samples from several *Crinum* plants growing at the US National Arboretum exhibiting mosaic symptoms tested positive for the presence of Potyvirus in an RT-PCR screen using several universal potyvirus primers yielding the expected 1,600 bp product corresponding to the partial N1b gene, full-length coat protein (CP) gene and 3'UTR. The RT-PCR amplicon was cloned and sequenced and NCBI BLAST analysis of the consensus sequence revealed highest similarities, but not identity, with isolates of NeYSV. Pair-wise analyses of the 258 amino acid sequence of the predicted CP had 65-68% sequence identity with the NeYSV isolates from *Crinum*, *Hymenocallis*, *Nerine*, *Stenomesson*, and *Vallota*; whereas the seven NeYSV isolates share 85-98% CP identity. We propose to associate this unique potyvirus sequence with the aforementioned CriMV.

#### **Viruses of brugmansia in Mississippi**

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*Brugmansia* (*Brugmansia* spp.), or Angel's trumpet, is an ornamental woody plant/shrub originating from South America and belonging to the family Solanaceae. Because of production of attractive large and fragrant flowers, they are now commonly grown in the temperate climate worldwide, including USA. As any other plant, it is susceptible to different pathogens including viruses. During the late summer/fall of 2015, several symptomatic brugmansia samples of different cultivars were brought to our attention by a local plant enthusiast. Symptoms varied among samples and, generally speaking, consisted of leaf mosaics and distortion. Samples were examined separately by biological, serological and molecular methods, including deep sequencing, and resulted in identification of several potyviruses, such as Colombian datura virus (CDV) and *Brugmansia mosaic virus* (BruMV). CDV was previously reported from brugmansia collected in Florida and few other states in the USA; however, this study represents the first state record for this virus in Mississippi. BruMV was not previously known to occur in the United States and was probably recently introduced into the country by infected plant material.

#### **Molecular Detection of Ti ringspot associated virus, a novel emaravirus associated with ti ringspot disease of *Cordyline fruticosa* (L.) in Hawai'i**

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An emerging virus-like disease of ti plant (*Cordyline fruticosa* L.) is spreading quickly throughout the Hawaiian Islands. Foliar symptoms include chlorotic lesions constricted by secondary veins or circular ring-spots that can coalesce into amorphous lesions. Tissue samples were collected from various locations across Hawaii and RNA was extracted. Degenerate RT-PCR primers targeting the polymerase gene of known emaraviruses amplified a ~400 bp product from the RNA of symptomatic plants. The RT-PCR products were sequenced and found to be >97.5% identical, indicating they represent a single virus species, designated Ti ringspot associated virus (TiRaV). When translated into amino acid sequences, this virus was found to be 70% identical to *Raspberry leaf blotch virus*, 55% to *European mountain ash ringspot-associated virus*, 53% to Redbud yellow ringspot virus, 50% to *Rose rosette virus*, 49% to *Fig mosaic virus* (FMV), and 46% to *Pigeon pea sterility mosaic virus*. These sequences and the *C. fruticosa* large rubisco subunit sequence were used to create specific primers and probes for a multiplex RT-qPCR detection assay. This assay was able to reliably detect TiRaV in symptomatic samples collected from various locations across Hawaii, and did not react to asymptomatic samples or cross react with an isolate of FMV.

#### **Is there resistance to Rose Rosette Disease among cultivated roses?**

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Rose Rosette Disease (RRD) is caused by the *Rose Rosette Virus* (RRV) and transmitted by a wind-blown, eriophyid mite (*Phylloctptes fructiphilus*). Little is known about resistance of roses to the virus or the mite vector. A few rose species, such as *Rosa palustris*, *R. setigera*, *R. carolina*, *R. virginiana*, and *R. spinosissima* have been reported to be resistant to RRD, but resistance in cultivated roses is unknown. Although it is possible to genetically introgress disease resistance from a wild species into the cultivated rose, the development of commercial roses would be quicker if there was a good source of resistance within the cultivated germplasm. Preliminary observational data on over 400 rose accessions indicates that there is resistance to RRD among the cultivated rose germplasm. Large field trials to assess RRD resistance of about 300 rose accessions have been planted in Tennessee and Delaware. The disease pressure in these trials is being augmented by inter-planting RRD infected roses among and by placing mite infested shoot tips from infected plants onto the plants to be tested for their resistance. In addition, smaller trials are being planted in Oklahoma and Texas to assess the level of RRD resistance of selected roses. Since disease symptoms may not appear for many weeks to months after inoculation with the virus, we anticipate this will be a long term study and it may be 1 or more years before we know if resistance occurs in cultivated roses.

#### **Survey for Grapevine Pinot gris virus infecting Grapevine in the Foundation Plant Services Vineyards at the University of California, Davis**

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Grapevine Pinot gris virus (GPGV) is a new member of the genus *Trichovirus* in the family *Betaflexiviridae*. The virus was first discovered by high-throughput sequencing from an Italian Pinot gris grapevine (*Vitis vinifera*) showing chlorotic mottling, leaf deformation and stunting symptoms. Since then, GPGV has been reported in symptomatic and asymptomatic plants of other cultivars in many countries. Recent transmission studies indicated that the eriophyid mite *Colomerus vitis* (Pagenstecher) is the suspected vector. This vector infests grapevine wherever they are grown and it has been reported in California. To address the possible presence and prevalence of GPGV in the collections of the Foundation Plant Services (FPS, University of California, Davis), 2,014 vines from the collection were screened including 23 vines of Pinot gris. Among all the vines tested, only one asymptomatic vine cv. Touriga Nacional was positive for GPGV. This grapevine cultivar has been imported from Portugal in 1981 and planted in the FPS collection in 2001. This is believed to be the first reported detection of GPGV in the U.S. Twenty samples in close proximity to the GPGV-positive vine were also collected and tested. These vines were all tested negative for GPGV. As a leading source of orchard and vineyard planting stock in the U.S., in addition to being a central component of the Grape Clean Plant Network, these negative results indicate that FPS stock is not a major source of GPGV.



### Identification and proposed species demarcation for *Cacao swollen shoot virus* and previously unidentified badnaviruses of *Theobroma cacao*

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Sustained production of cacao (*Theobroma cacao*) in West Africa, which supplies over 70% of the world's bulk cacao, is hindered by virus-like diseases. To date, *Cacao swollen shoot virus* (CSSV) (*Caulimoviridae*, *Badnavirus*), endemic to West Africa, is the only known cacao-infecting badnavirus. In West Africa, CSSV-like symptoms became prevalent during 2000-2003, and continue to persist. Since the 1940s, virus-like symptoms in cacao of suspect badnaviral etiology have been recognized in Trinidad at the International Cocoa Genebank, one of the custodians of cacao germplasm resources. To identify the suspect viruses, total DNA isolated from symptomatic cacao leaves collected in Ghana, Cote d'Ivoire and Trinidad was subjected to rolling circle amplification and sequenced using Illumina HiSeq. DNA sequences were assembled using DNASTAR, and annotated using the NCBI ORF Finder, and the results were validated by sequencing PCR-amplified full-length genomes. Genome characterization revealed seven previously unidentified badnavirus isolates, each with four open reading frames, characteristic of other badnaviruses. Pairwise comparisons using Standard Demarcation Tool (SDT) of the RT-RNaseH sequence (80% nucleotide identity) with analogous badnavirus sequences indicated that the isolates represented three new badnavirus species. However, SDT analysis of the full-length genome sequences suggested at least five previously unidentified badnavirus species are associated with cacao.

### First report and characterization of *Taro bacilliform virus* in the USA

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*Taro bacilliform virus* (TaBV) is a member of the genus *Badnavirus* (Family *Caulimoviridae*) and a pathogen of taro (*Colocasia esculenta*), an important starch staple in tropical regions. TaBV is transmitted by mealybugs and appears to be widely distributed throughout the tropical Pacific. Taro has cultural, economic and nutritional importance in Hawaii, but only *Dasheen mosaic virus* and *Taro vein chlorosis virus* have been reported in the state. We surveyed 328 taro plants from 35 sites on the islands of Kauai, Oahu, Molokai, Maui, and Hawaii for the presence of TaBV by PCR using degenerate badnavirus and TaBV-specific primers. Of these, 171 samples (52%) tested positive using the degenerate primers, but only 153 tested positive using TaBV-specific primers (47%), indicating the presence of novel badnaviruses and/or strains of TaBV in Hawaii's taro. The 7825 bp genome of one isolate of TaBV was sequenced and found to be 80% identical and 367 bp larger than the only complete TaBV genome in GenBank (accession AF357836), indicating that considerable genetic diversity exists for this virus. PCR, RT-PCR and Southern hybridization assays were conducted and provided evidence that TaBV is integrated into the genome of some Hawaiian taro varieties. TaBV was mechanically inoculated onto eight herbaceous plant species, and after 25 days, TaBV could be detected by PCR in *Cucurbita pepo*, *Pisum sativum*, *Cucumis sativus*, *Vigna unguiculata*, and *Phaseolus vulgaris*.

### Association of a mixed infection of *Lettuce chlorosis virus*, *Papaya ringspot virus*, and *Tomato yellow leaf curl virus-IL* in a Texas papaya orchard

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Severe virus-like symptoms consisting of mosaic, distortion, yellowing, and brittleness were observed on papaya plants in a 50-ha orchard in South Texas during the 2014-2015 growing season. Disease incidence increased from ~40 to 100% within six-months of the outbreak and severely affected plants were stunted with reduced fruit loads and poor fruit quality. cDNA library constructed from DNase-treated total nucleic acids from symptomatic plants was subjected to high-throughput sequencing (HTS). Based on BLASTn analysis of the assembled HTS contigs, the plant viruses detected were validated by Sanger sequencing of the complete genome sequences. The collective sequencing results indicated the presence of *Papaya ringspot virus* (PRSV; *Potyvirus*), *Lettuce chlorosis virus* (LCV; *Crinivirus*), and *Tomato yellow leaf curl virus* (TYLCV-IL; *Begomovirus*). RT-PCR analyses of leaves from 51 randomly sampled papaya plants indicated the presence of PRSV, LCV, and TYLCV-IL in 100, 39.2 and 15.7% of the samples, respectively. Plants with mixed infections of PRSV, in combination with LCV and/or TYLCV-IL, exhibited more severe symptoms compared to plants with PRSV alone. The association of LCV and TYLCV-IL with symptomatic plants represents first report of these whitefly-transmitted viruses in papaya. Papaya, a perennial crop, could serve as an over-seasoning reservoir for TYLCV-IL and LCV presenting new challenges to disease management in papaya and other crop hosts of these viruses.

### Seed-borne virome in cucurbits

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In this work we investigated the 'community' of seed-borne viruses in more than 50 cultivars and PI lines/accessions belonging to different botanical species of cucurbits by the next-generation sequencing approach. To this aim, double stranded RNAs (dsRNAs) were extracted from plant tissue collected at the first true leaf stage from a pool of young seedlings belonging to the same genotype and grown under greenhouse conditions in order to prevent contamination by horizontally transmitted viruses. Polyacrylamide gel electrophoreses (PAGE) revealed the presence of single or multiple dsRNA bands in the majority of tested plants indicating ongoing infections by vertically transmitted viruses. The dsRNA extracts were reverse transcribed, tagged, and submitted to Illumina sequencing. Results of the sequence analyses demonstrated the widespread presence of persistent viruses in tested cucurbits, mainly those belonging to the families *Partitiviridae*, *Amalgaviridae*, *Endornaviridae* and *Totiviridae*. In this study, we identified and characterized numerous viruses, representatives of new species and confirmed the prevalence of *Cucumis melo* endornavirus and *Lagenaria siceraria* endornavirus in tested melons and bottle gourds, respectively. The importance of widespread presence of persistent viruses in some cucurbits and their possible effects to the host are yet to be understood.

### Reemergence of the torradovirus, *Tomato necrotic dwarf virus*, in processing tomatoes in the Central Valley of California

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In the fall of 2015, processing tomato plants (*Solanum lycopersicum*) in a late-planted field in Kern County, California had high populations of whiteflies and developed virus-like symptoms including stunting and distorted growth; leaf curling, crumpling and necrosis; and stem and shoot necrosis. The symptoms resembled those induced by *Tomato spotted wilt virus* (TSWV), but immunostrip and RT-PCR tests were negative for TSWV and other tospoviruses. RT-PCR tests were then performed for *Tomato mosaic virus* (ToMV), *Alfalfa mosaic virus* (AMV), *Tomato necrotic spot virus* (ToNSV),

and torradoviruses (family *Secoviridae*) were performed. The expected size DNA fragments were amplified for ToMV and torradovirus (degenerate primers for RNA-1 and RNA-2), whereas negative results were obtained for AMV and ToNSV. Analysis of the DNA sequences of the PCR-amplified torradovirus RNA-1 and RNA-2 fragments revealed that these plants were infected with a torradovirus that was most similar (~94%) to RNA-1 and RNA-2 of *Tomato necrotic dwarf virus* (ToNDV). This is the first report of ToNDV in the Central Valley of California, although it was previously reported from the Imperial Valley of California in the 1980s. Surveys to determine inoculum sources and further sequencing and biological characterization of ToNDV isolates in California are in progress.

#### **Discussion on calculation of disease severity index values from scales with unequal intervals**

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When estimating severity of disease, a disease interval (or category) scale comprises a number of categories of known numeric values – with plant disease this is generally the percent area with symptoms (e.g., the Horsfall-Barratt (H-B) scale). Studies in plant pathology and plant breeding often use quantitative interval scales. The severity is estimated as an interval scale value and is used to determine a disease severity index (DSI). However, very few studies have investigated the effects of different scales on accuracy of the DSI. Using an interval scale designed for pecan scab (*Fusicladium effusum*), we investigated performance of the DSI. We used interval scale values, the scale data converted to midpoint values of each category, and nearest percent estimates (NPEs). Results of the pecan scab data indicate that there is a consistent bias of the mean value when applying the interval scale values directly. The mean value of the DSI per cultivar is underestimated as compared with that of NPEs; results of the mid-point conversion are akin to those of the NPEs. Furthermore, based on the data used, the simulation studies were executed to explore the mean value of DSI for three different parameters. The results of these simulation studies are consistent with those of studies which used real data. Finally, a new disease index, which uses equal intervals with additional grades at low severities, was developed to minimize the effect of the scale on error.

#### **Predicting Robust Candidates for a Boxwood Blight Immunoassay: An Automated Computational Workflow with Broad Applications for Phytopathology**

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The boxwood blight fungal pathogens *Calonectria pseudonaviculata* and *C. henricotiae* have emerged as a serious threat to natural and cultivated plant populations around the world. These pathogens can persist as latent infections in asymptomatic plant tissue even after fungicide treatment. Therefore, a fast and field-deployable diagnostic test based on immunological detection would provide a useful tool to help minimize spread of the pathogen. Although several nucleic acid-based approaches are available to detect boxwood blight pathogens, none are field-deployable solutions. We are developing an integrated computational pipeline using recent genomic and RNA-Seq data to identify quality candidate sequences for use in designing a diagnostic immunoassay. The pipeline first identifies proteins likely to be secreted using SECRETOOL. To robustly test the putative secreted proteins for solubility, we developed a new classifier with improved recognition performance over other methods using a publicly-available dataset of secreted proteins. The candidate proteins are further narrowed down through a comparative genomics analysis that identifies proteins with predicted antigenicity that are specific to the target pathogens yet share low similarity to outgroup species. This pipeline stands to help speed up immunoassay development and lower costs by providing a starting set of candidate proteins with a higher likelihood of success in further wet laboratory experiments.

#### **Identification of conducive temperatures for decision support modeling of sugarcane rusts in Florida**

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Most sugarcane varieties grown in Florida are currently susceptible to either brown (*Puccinia melanocephala*) or orange (*Puccinia kuehnii*) rust, two diseases that have been reported to cause economic losses in sugarcane industries worldwide. In Florida, sugarcane rusts are controlled using fungicides and application schedules are solely based on disease scouting. In order to develop a forecasting system to optimize fungicide applications, disease severity data were collected during the entire 2014 crop season. Percent rust affected area of the top visible dewlap leaf was recorded every two weeks from seven sugarcane varieties susceptible to brown or orange rust and planted in October, November and December 2013. Brown and orange rust progress curves were generated using maximum disease severity values at each scoring date, whatever variety and planting date. Because relative humidity and leaf wetness does not appear to be a limiting factor for development of sugarcane rusts in Florida, efforts were focused on temperature that is also known to affect rust progress. Curves of cumulative average daily temperatures between 17°C and 29°C were compared with disease progress curves. Temperature ranges of 17-25.5°C and 17-26.5°C appeared to be the best fit for progress of brown and orange rust, respectively. Weather and disease data from additional crop seasons need to be analyzed to confirm these first results and to develop a decision support model for rusts in Florida.

#### **Comparing epidemics of unique *Phytophthora infestans* clonal lineages with biometrically enhanced spatio-temporal modeling tools**

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The oomycete *Phytophthora infestans* causes late blight, a plant disease notorious for giving rise to the Irish Potato Famine of the 1840s. In 2009, a pandemic caused by a newly emerged clonal lineage of *P. infestans* (US-22) destroyed gardens and small farm tomatoes and potatoes across the US. In the following years, three clonal lineages (US-22, -23, -24) comprised national epidemics, with US-23 predominating from 2013 onward. US-23 is of noteworthy aggressiveness, has small sporangial size compared to previous lineages, and is highly pathogenic on both potato and tomato. Previously, two disease dispersal models were fit using Markov chain Monte Carlo (MCMC) for late blight epidemics in central Wisconsin caused by clonal lineage US-8. In our current work, we validated and enhanced these models using the late blight epidemic caused by US-23 in this region for 2009-2015 and we fit a spatial-temporal semivariogram for both epidemics. Disease data were collected from scouting records of a cooperating crop consultant and were manipulated using ArcGIS and RStudio. The power law model predicted disease dispersion accurately with  $R^2$  values between 0.2 and 0.9. Sporangial size greatly influenced dispersion rate which played a role in disease progression. Modeling of specific clonal lineage epidemics in high-risk production regions can aid in developing prescriptive, risk-averse late blight management plans to reduce disease perpetuation and economic loss.

#### **Impacts of climate change on wheat powdery mildew epidemics in China**

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Epidemics of wheat powdery mildew (*Blumeria graminis* f. sp. *tritici*) have been highly destructive to winter wheat in China. We analyzed trends in the regions of wheat powdery mildew epidemics in China from 1970 to 2012 and PA (percent area affected by wheat powdery mildew) in response to climate change. Using PA and temperature for pathogen overwintering and overwintering periods, a model was constructed and subsequently used to predict changes in PA for 15 Global Climate Models under low (B1), medium (A1B), and high (A2) climate emission scenarios for the 2020s, 2050s, and 2080s relative to the 1970–2009 period. Our results showed a significant increase in T (mean monthly air temperature) ( $P < 0.001$ ), while SH (sunshine hours) and RH (relative humidity) decreased. Using the original value method, there was a significant positive relationship between T and PA ( $P < 0.05$ ). Using the first-differences time-series value method,  $\Delta T$  (first-differences in T) in overwintering and late spring periods were negative for  $\Delta PA$  (first-differences in PA) while positive relationships were recorded in the rest of the periods. The results using the first-differences time-series value method

were more precise for disease epidemics. The results of model prediction indicate that the values for the percent area affected by powdery mildew will increase in future. These findings will aid in decision making for management strategies of wheat powdery mildew.

#### **Quantifying the potential effects of regional climate change on wheat leaf rust disease in the Grand-Duchy of Luxembourg**

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Climate change will affect wheat crop production both in the main processes of plant growth and development and in the occurrences and severities of plant diseases. We assessed the potential infection periods of wheat leaf rust (WLR) in a changing climate at two climatologically different sites in Luxembourg using a threshold-based model for WLR. Field experiments were conducted during 2003–2013 at the selected sites to test the model. Projected climate data, from a multi model ensemble of regional climate models (spatial resolution 25 km) as well as an additional projection with a higher spatial resolution of 1.3 km, were used to investigate the potential WLR infection periods for two future time spans. The model simulated satisfactorily WLR infection periods at both sites during 2003–2013: probabilities of detection were close to 1 and the critical success index ranged from 0.80 to 0.94. Regional climate projections indicate an increase in temperatures over the 2014–2050 and 2091–2100 periods compared to the reference period 1991–2000. Annual precipitation is also expected to increase slightly in the future. Moreover, trends in increased favourable days of WLR infection would likely occur at both sites due to projected climatic conditions more conducive than in the reference period. Our methodology can be easily transferred to other regions and other fungal diseases by adjusting the meteorological threshold values essential for a specific disease development.

#### **Screening some rust universal-susceptible wheat varieties for ozone tolerance and some applications**

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Elevated ground-level ozone associated with climate change and the rapidly evolving rust races are major challenges to wheat production worldwide. Because of higher susceptibility, rust universal-susceptible varieties are used as susceptible hosts to maintain and increase inoculum for most of the rust races. These varieties could also be used to study the interactive effects of ozone on different rust races. A first step is to assess the O<sub>3</sub> responses of these wheat varieties. In this research, we screened the wheat lines Chinese Spring, CSA, Line E, Little Club, LMPG-6, McNair 701, Morocco, Rusty and Thatcher for O<sub>3</sub> tolerance at four O<sub>3</sub> concentrations. Results showed that Thatcher is the most sensitive and Chinese Spring is the most tolerant among the nine lines tested. For future studies of the effects of ozone on different stem rust races, LMPG-6, Rusty and McNair 701 could be used as O<sub>3</sub>-sensitive if compared to the more ozone-tolerant CSA or Chinese Spring. Similarly, Thatcher is a suitable O<sub>3</sub>-sensitive line that could be used with either Little Club or Morocco for studying O<sub>3</sub> effects on different races of leaf rust. Identification of lines having differential ozone response will enable further genetic studies on ozone tolerance.

#### **Distribution models of common bean dry root rot in Brazil: Current and predicted disease distribution in climate change scenarios**

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Dry root rot is an ubiquitous problem on common beans in Brazil, with yearly yield losses attributed to the *Fusarium solani* species complex. Species distribution models (SDMs) based on 21 field disease records were developed to assess dry root rot current geographical distribution, and estimate disease range expected in IPCC scenarios A1B and A2 by the year 2030. Projections were adjusted by an ensemble of three modelling methods: Generalized Additive Models, Surface Range Envelop and Artificial Neural Networks, built with “biomod” and “raster” packages from R statistical software. Also, we verified if three inoculum thresholds (1200, 3700 and 4500 propagules of *F. solani* per soil, in 2623 records) could work as disease spatial proxies, supported by Sperman’s correlations between inoculum thresholds and disease records. The best disease × soil inoculum density correlation was found with 3700 propagules per soil gram ( $r=0.85$ ,  $P<0.001$ ). The current projection shows high climatic suitability for common bean dry root rot in the Brazilian central region. According to IPCC scenarios, the geographical area affected by the disease will be reduced by almost 23%, but highly favorable areas will move towards Brazil South Region, which represents 40% of the country’s common bean yields. Implications and consequences of these results will be discussed to support future plans on root rot management.

#### **Discovery of biological drivers of pitch canker disease in a changing climate**

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Natural and agricultural ecosystems are affected by climate change through increased temperature, humidity, and unusual weather patterns. They are increasingly threatened by invasions by non-native pests and pathogens. The fungus *Fusarium circinatum* causes pitch canker disease in several pine species, leading to high economic losses in the timber industry. Favored by high temperatures and humidity, future outbreaks may become more frequent under predicted changes in environmental conditions over the next 50–100 years. A better understanding of *F. circinatum* biology is needed to inform predictions of which pathogen variants would likely cause outbreaks in future climate settings. We evaluated growth, spore production and germination among *F. circinatum* isolates cultured at various temperatures (25, 28, and 31 °C). We also implemented spore trapping experiments in three north-Florida sites to survey spore release throughout the spring and summer, and to test if weather events trigger increased spore discharge. Results show significant temperature effects ( $p<0.05$ ) in mycelium growth and sporulation among isolates. Though spore trapping experiments need to be followed across several growth seasons, we present preliminary trends on spore release in the environment. These results will constitute the baseline for future research on fungal diversity, spatial distribution patterns and disease prediction, aimed at mitigating adverse effects of climate change on forest health.

#### **Potential species distributions of *Armillaria solidipes* and *Pseudotsuga menziesii* under contemporary and changing climates in the interior western USA**

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*Pseudotsuga menziesii* and *Armillaria solidipes* are native forest host/pathogen species of the interior western USA that have co-evolved over millions of years. *Armillaria* root disease caused by *A. solidipes* is a major limitation for growth, survival, and economic/environmental benefits of *P. menziesii*. Historically, *A. solidipes* played a beneficial ecological role by preferentially attacking maladapted trees. However, under increasing climate change, more forest trees will likely become progressively maladapted, leading to increased damage from *Armillaria* root disease. We developed species-

distribution models using Maximum Entropy (MaxEnt) to predict area suitable for *A. solidipes* and *P. menziesii* under changing climatic conditions. We compared contemporary (1950-2000) predicted suitable climate for *A. solidipes* and *P. menziesii* versus year 2070 (2061-2080) predictions using the “business as usual” greenhouse gas-emissions pathway 8.5 (RCP8.5) paired with the HadGEM2-ES global circulation model. Our predictions of suitable climate for *A. solidipes* and *P. menziesii* are highly correlated, and the predicted suitable climate space moves dramatically northward for year 2070 under the RCP8.5 scenario. In many areas of the inland west, *A. solidipes* is predicted to persist under climate change, and it will likely cause major damage where maladapted forest trees remain.

#### **Effects of vernalization on the differential ozone response of two winter wheat genotypes**

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Elevated ground-level ozone, associated with climate change is a limiting factor to wheat production. Evaluating winter wheat for ozone responses could be accomplished more rapidly at the seedling stage using unvernialized seedlings. However, there is no information available on the effects of vernalization on the expression of ozone injury responses, and the association of these responses to yield losses at adult plant stage. Twenty five winter wheat genotypes (including historical and recent varieties and breeding lines) were evaluated for ozone tolerance (at CF, 50, 70, 90 and 110 ppbv, 8hrs/day for 1 week), and two lines with differential ozone response were selected, Coker-9553 (O3-sensitive) and MD01W28-08-11 (O3-tolerant). These two genotypes were used to study the effects of vernalization on the differential ozone response, and the validity of results if unvernialized seedlings are used. Foliar Injury results at the seedling stage showed that vernalization has no significant effect on the differential ozone responses, and the two genotypes could be separated using vernalized or non-vernalized plant material. The differential response obtained at the seedling stage was validated at the adult-plant stage. Yield losses under elevated ozone treatments in open-top chambers were associated with increased foliar injury observed at the seedling stage.

#### **Screening the monosomic lines of the Chinese Spring wheat variety for ozone tolerance**

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Elevated tropospheric ozone is currently a major challenge to wheat production worldwide and is anticipated to be more challenging in the future. Breeding wheat for ozone tolerance is needed to maintain or increase wheat yield. However, there is limited information on the genetic control of ozone tolerance in wheat. The current understanding is that genome A and genome B are responsible for tolerance, whereas genome D is responsible for ozone sensitivity of modern wheat. In this study, we investigated the contribution of each of the 21 chromosomes of the Chinese Spring variety towards its ozone tolerance. We screened the 21 monosomic lines of Chinese Spring, at four ozone concentrations (CF, 50, 70 and 90 ppbv, 12 hr daily average, for 2 weeks). Ozone-induced foliar injury results showed that the absence of chromosome 7A reduces ozone tolerance. In contrast, the absence of chromosome 4A increases tolerance. None of the chromosomes of the genome D were found to individually reduce ozone tolerance. Future studies are needed to identify the genetic basis of ozone tolerance and the associated DNA markers to facilitate the incorporation of ozone tolerance into wheat breeding programs.

#### **Spatio-temporal brown eye spot progress of irrigated coffee by central pivot**

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The brown eye spot of coffee (*Cercospora coffeicola*), reduces both productivity and quality of the beverage. Thus, the objective was to evaluate the spatio-temporal disease progress in coffee farm irrigated by center pivot. The pivot area, located in the southern state of Minas Gerais in Brazil, was georeferenced with 50 points in 17 ha to proceed the geostatistical analysis. The disease incidence was evaluated in 60 leaves, in five plants/point at 60-days intervals, from August 2012 to December 2014, totaling 15 evaluations. Climatic variables were obtained by weather station installed in the area and the data were correlated with disease incidence. The production of the years 2013 and 2014 were correlated with the area under the incidence progress curve (AUIPC). The disease progress curves were plotted and the adjustment of semivariogram models was done. Then, the data were interpolated by ordinary kriging, and the maps of disease were constructed (ArcGIS 9.2®). The incidence reaching in August 2013 and 2014, 23 and 21%, respectively. The exponential model was adjusted to the semivariogram. In over 50% of the area, the intensity was high, exceeding 20%, with spatial gradient. There was negative correlation between incidence of the disease with minimum and medium temperatures and AUIPC and productivity in 2014.

#### **Effect of pre-anthesis rainfall patterns on fusarium head blight and deoxynivalenol in wheat: A multi-state study**

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Although there is often a correlation between visual symptoms of Fusarium head blight (FHB) and deoxynivalenol (DON) in wheat, under certain conditions, DON levels may be higher than one would expect based on visual symptoms. Between 2012 and 2015, field experiments were conducted at three locations (Minnesota, North Carolina, and Ohio), representing different wheat growing regions and market classes, to investigate the effects of intermittent rainfall during the 7-8-day pre-anthesis window on FHB and DON in wheat. Plots were inoculated with corn kernels colonized by *Fusarium graminearum* at jointing (Feekes GS 6). The experimental design was a randomized complete block with five pre-anthesis simulated rainfall treatments: 1) rainfall every day (Rain\_1); 2) rainfall only on the first and last two days of the window (Rain\_2); 3) rainfall only on the middle 3-4 days of the window (Rain\_3); 4) rainfall every other day (Rain\_4); and 5) check. Plots that received simulated rain had numerically higher mean FHB incidence (INC), index (IND), Fusarium damaged kernels (FDK), and DON than the check at all locations. Rain\_1 consistently resulted in higher mean INC and IND than Rain\_2, Rain\_3 and Rain\_4. However, Rain\_2 and Rain\_4 had comparable mean FDK and DON to Rain\_1. Thus, dry-wet moisture cycles may be associated with disproportionately high DON for a given level of IND.

#### **Changes in frequency of *Leptosphaeria maculans* avirulence genes in North Dakota**

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Blackleg, caused by *Leptosphaeria maculans* is resurging as a threat to North Dakota canola industry. A shift in the virulence profile of *L. maculans* populations is thought to be responsible for it. Estimating the prevalence of specific avirulence genes in *L. maculans* populations could be used to infer the relative value of its interacting resistance genes for a plant breeding program. The objective of this study was to determine the frequency of avirulence genes in *L. maculans* isolates from North Dakota. Single-spore cultures of *L. maculans* isolates collected from commercial grower's fields in 2004, 2007 and 2014 were used. Presence of avirulence genes *AvrLm1*, *AvrLm6*, *AvrLm4-7* and *AvrLm11* was determined using PCR assays. The results indicated that the frequency of *AvrLm1* increased from 3% among isolates collected in 2004 to 45% among isolates collected in 2014 while the frequencies of *AvrLm6* and *AvrLm11* remained unchanged (91 to 100% and 91 to 95%, respectively) between the same two periods. *AvrLm4* and *AvrLm7* alleles were present in 90 and 100% of the isolates, respectively in 2014. Determination of frequencies of *AvrLm4* and *AvrLm7* in 2004 and 2007 is underway. The higher frequency of isolates carrying *avrLm1* in 2014 suggests its corresponding resistance gene, *Rlm1*, can no longer provide effective

protection. In contrast, the high prevalence of avirulence genes *AvrLm4*, *AvrLm6*, *AvrLm7*, and *AvrLm11* indicates their interacting resistance genes could be safely used.

#### **Transmission of *Rhodococcus* spp. causing pistachio bushy top syndrome in California**

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Pistachio bushy top syndrome (PBTS) is a new pistachio disease caused by two *Rhodococcus* spp., highly related to *Rhodococcus fascians* (*Rf*) and *Rhodococcus corynebacterioides* (*Rc*). Determination of potential modes of pathogen transmission is necessary for protection of replants and asymptomatic trees in orchards, as well as prevention of similar future epidemics. The objectives of this study were to assess the potential for PBTS isolates to be transmitted to healthy plants via naturally-infested soil at replant sites, using pruning tools, or by rodents. Clonal UCB-1 (*Pistacia atlantica* x *Pistacia integerrima*) plants were planted in naturally-infested soil; positive and negative control treatments were established by placing root-inoculated and uninoculated plants in steam-disinfested soil. Pruning transmission studies were conducted by cutting into symptomatic tissue in advance of cutting healthy plants; positive and negative control treatments were established by artificial infestation and flame sterilization of pruners, respectively. Mice trapped in the greenhouse were evaluated for internal and external populations of *Rf* and *Rc*. The results demonstrate that naturally infested soil and infested pruning tools may transmit the bacteria to healthy plants. Mice were also found to carry *Rhodococcus* sp. on fur and in their digestive tract. The results underscore the need for vigilant sanitation, both in the orchard and in nursery propagation systems.

#### **Quantification of airborne inoculum of *Magnaporthe oryzae* by using TaqMan Real-time PCR**

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Rice blast caused by *Magnaporthe oryzae* is one of the most devastating diseases in China and worldwide. Accurate and timely quantification of airborne spores of the pathogen is important to disease forecast and management of the disease. In this study, airborne spores were trapped on the tape with a Burkard sporewatch spore samplers mounted in a rice field in Panjin, Liaoning province, China from July 1 to October 13, 2015. The spores were washed off the tape and followed by DNA extraction by using Mobio Power Soil DNA Isolation Kit, and quantified by TaqMan Real-time PCR. Specific primers and probe were designed to target the 28S rDNA of *Magnaporthe oryzae* and reaction system was optimized. qPCR results of field samples revealed two sporepeaks during the study period. They were 9.46 spores /m<sup>3</sup> from July 18 to 20 and 2.95 spores /m<sup>3</sup> from August 15 to 17, which were followed by a high incidence of leaf blast on August 4 and first observation of neck blast on August 22. This suggested that quantity of airborne spores of *Magnaporthe oryzae* could be useful in disease forecasting of rice blast. The qPCR system combined with spore samplers provided an efficient way to quantify airborne pathogen for disease forecasting and guiding disease management of rice blast.

#### **The seasonal dynamics of *Phyllosticta* spp. in Floridian citrus grove leaf litter**

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The causal agent of Citrus Black Spot (CBS), *Phyllosticta citricarpa*, is a restricted pathogen in Europe and quarantined in the US after it was found in Florida in 2010. Additionally, an endophytic fungus *P. capitalensis*, associated with a wide range of hosts including citrus, is globally distributed and commonly found in Floridian citrus. These two species cannot be distinguished morphologically; therefore, previous CBS epidemiology studies included both endophyte and pathogen. Both fungi overwinter and produce ascospores and conidia in leaf litter. Understanding the seasonal dynamics of these two species in leaf litter can provide knowledge to control CBS inoculum in Floridian groves. 1000 leaves were collected biweekly from 40 arbitrarily selected trees in a grove in south Florida since May 2014. The number of leaves with fructifications, the number of *Phyllosticta* spores collected from the leaf litter, and the average leaf degradation level from each sample were recorded. The quantity of *P. citricarpa* and *P. capitalensis* DNA in leaf litter and spore suspensions was determined via qPCR. The results showed that the fructification abundance is correlated with leaf degradation level (pseudothecium:  $P = 0.0008$ , pycnidium:  $P = 0.0333$ ), conidia were always present, and ascospores were occasionally absent. The quantity of *P. capitalensis* DNA was constant while the quantity of *P. citricarpa* DNA increased throughout the two-year sample collection period.

#### **Field observations of ascospore discharge of *Monilinia vaccinii-corymbosi* in northern highbush blueberries**

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*Monilinia vaccinii-corymbosi* (*Mvc*), is the causal agent of a disease known as mummy berry in highbush blueberries (*Vaccinium corymbosum* L.). The primary inoculum of *Mvc* is discharged from apothecia grown from mummified blueberry fruits that fall to the ground in previous seasons. In order to better predict the release of primary inoculum a study of ascospore discharge was conducted using aerial spore trapping and several weather variables. A Burkard spore trap was used to collect ascospores within infested blueberry fields in 2002, 2003, 2004, and 2009. A previously developed degree-day model was also assessed in order to compare the amount of chill-hours and degree-days required for southwest Michigan grown blueberries. Peak ascospore discharge was inversely related to daily relative humidity and the majority of ascospores were discharged between 67-107 growing degree-days and after 3000 chill-hours were attained. Additionally, peak ascospore discharge was reached when apothecial cup size was between 2-7mm in diameter which correlated with previous laboratory studies. The information in the study may be used to better understand the conditions needed to predict mummy berry apothecia emergence and ascospore discharge with the aim of improving future disease management practices.

#### **A leaf litter and fruit brown rot life style of *Phytophthora syringae* in California citrus**

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In recent years, *Phytophthora syringae* has become a major pathogen causing brown rot of citrus fruit in California during the winter season. This is a serious concern for the citrus industry because *P. syringae* is a quarantine pathogen in some export countries. The source of *P. syringae* inoculum infecting citrus fruit has never been clearly established. Roots and rhizosphere soil from trees where brown rot was found to be caused by *P. syringae* were sampled in three orchards. Samples were plated onto a *Phytophthora*-selective medium and TaqMan qPCR and Droplet Digital PCR were performed. *P. citrophthora* and *P. parasitica* were consistently detected by isolation in roots and soil and by PCR in roots. *P. syringae* was not found causing root rot and was not detected in the soil using these methods. Leaf litter on the ground and soil were sampled from the same locations and immersed in water. Mature green pears were used as baits and incubated at 12°C for 1 to 2 wk. Brown lesions that developed on pears at the water line were plated onto selective medium. *P. syringae* was detected in most leaf litter samples but only in two soil samples. Thus, leaf litter is a major inoculum source for *P. syringae* and changes our understanding of the disease cycle of this important pathogen and separates it from other *Phytophthora* species that cause citrus root and brown rots. Recognition of the distinct epidemiology of the disease opens the opportunity for new cultural management practices.

**Specific TaqMan qPCR and a PvABC qPCR assays for monitoring airborne sporangia of *Plasmopara viticola* f. sp. *riparia*, *aestivalis* and *vinifera***  
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Grape downy mildew (*Plasmopara viticola*) is among the most important grapevine diseases in eastern Canada. Recently, five new cryptic formae speciales of *P. viticola* from wild and cultivated *Vitis* species were described. In Eastern Canada, only *P. viticola* f.sp. *riparia* (clade A) and *P. viticola* f.sp. *aestivalis* (clade B) have been detected. As part of a biovigilance network aiming at tracking new pathogens and new genotypes, molecular assays for simultaneous quantification of sporangia of clade A and B and detection of clade C (*P. viticola* f. sp. *vinifera*) from air samples were developed. The assays are a specific triplex TaqMan qPCR and a PvABC qPCR developed from sequence alignments of internal transcribed spacer 1 and 2 (ITS1-2) DNA regions for *P. viticola* clade A, B, C and other closely related species. These assays were used to study the temporal and spatial occurrence of these genotypes in two vineyards. At one site, almost exclusively (99%) clade A was found while at the other site it was mostly (83%) clade B. Hence the genotype A was found at both sites, however, it was observed for the first time in early July at one site and in early June at the other site. The impact of these genotypes on grape downy mildew development is under study. Using these new tools, it will be possible to acquire knowledge on aerobiology and spatio-temporal distribution of genotypes A and B and to monitor clade C not present in Eastern Canada, but detected in north-east of USA.

**First report of the Asian citrus psyllid, vector of *Candidatus Liberibacter asiaticus* in Tanzania and their potential further spread in Africa**

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Citrus surveys were conducted at high (>700m), medium (300-600m) and low (<200m) altitudes in Tanzania. Adults and nymphs of *Trioza erythrae* were abundant in the highlands and less abundant at medium altitudes. Unexpectedly, adults and nymphs of *Diaphorina citri*, the Asian citrus psyllid, were found at medium altitudes, around Morogoro. No psyllids were observed at low altitudes. Severe huanglongbing symptoms and tree decline were evident at high altitudes, while mild symptoms were observed at intermediate and low altitudes. DNA was extracted from leaf and psyllid samples and subjected to conventional PCR with seven different primer sets and qPCR with two primer sets for *Candidatus Liberibacter* spp. PCR products were sequenced and subjected to phylogenetic analysis. *C. Liberibacter africanus* (Laf) was detected in leaf and *T. erythrae* samples from high and medium altitudes by all methods. Sequences of *C. Liberibacter* DNA from leaves and psyllids were similar to those of Laf from South Africa. Even though Las primers gave a positive reaction in qPCR with medium altitude leaf samples, cPCR and sequencing results were negative for Las, suggesting cross reactivity of Las primers with Laf. Neither Laf nor Las were detected in *D. citri* samples. This is the first report of *D. citri* in Africa. Further surveys are needed to detect Las. The potential distribution of *D. citri* and Las in Africa was predicted using the correlative models MAXENT and Multi-Model Framework.

**Genetic diversity of pathogenic *Clavibacter michiganensis* subsp. *michiganensis* strains from Central Chile**

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The bacterial canker of tomato caused by *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) is the most important bacterial disease in tomato. The genetic diversity of *Cmm* strains isolated during 20 years from tomato plants in different Chilean tomato regions was studied. Twenty five isolates were characterized by growth on different media, Gram staining and enzymatic activities. For bacterial identification PSA8/R PCR amplification and 16S rRNA gene sequencing were used. In addition, the housekeeping *gyrB* and *kdpA* genes and the pathogenicity *tomA* and *chpC* genes were sequenced. All genes were polymorphic in these isolates. The *kdpA* gene showed the highest variability. Except for the *kdpA* gene, Chilean isolates genes showed high identity with genes from reference strain NCPPB382. Phylogenetic trees of *gyrB* and pathogenicity genes showed close relationship between Chilean strains and those from New York and Serbia. Analyses of concatenated *kdpA-gyrB* genes yield 7 Chilean haplotypes. Geographical distribution of the isolates was analyzed. Most of the Chilean isolates clustered separately from the Serbian and New Yorker strains, suggesting that Chilean bacteria have a different origin. Additional housekeeping genes and a higher number of isolates will be studied.

**Spatiotemporal analysis of wheat blast epidemiology (*Magnaporthe oryzae* Triticum pathotype) under natural field conditions**

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Wheat blast is an emerging fungal disease of wheat caused by the Triticum pathotype of *Magnaporthe oryzae*. In years when weather is conducive for disease development, growers can suffer total crop losses. In order to understand the epidemiology of wheat blast, it is important to characterize the pattern of disease spread within a field. This pattern provides clues about the characteristics of the pathogen that affect the spatiotemporal dynamics of the disease. In this study, inoculum build-up and temporal and spatial spread from naturally occurring in-field disease foci (hotspots) were investigated in six 27 × 27 m<sup>2</sup> plots at three locations in Bolivia during the 2015 growing season. Plots were assessed for spike blast incidence and severity every 3 days. Assessments were made on a grid, with twenty spikes rated every 2.7 m. Hotspots increased in size over time and were associated with the highest disease ratings. Initially, plants adjacent to the hotspots had higher disease levels than plants located farther from the hotspots. As the disease progressed the steepness of the disease gradient decreased away from hotspots, and high levels of incidence and severity were observed throughout the plots. This trend was observed across the three locations. Preliminary spatial analyses suggest that hotspots are important in-field sources of inoculum.

**A Snapshot of Citrus Black Spot in Southwest Florida: Spatial Distribution in Two ‘Valencia’ Citrus Groves**

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Citrus black spot (CBS) an emerging disease of citrus in Florida caused by the fungus *Guignardia citricarpa* Kiely (anamorph *Phyllosticta citricarpa* (McAlpine) van der Aa) was identified in March 2010. The source of the introduction into the United States is still unknown. Disease inoculum, conidia and ascospores are dispersed by water and wind respectively. Due to quarantine restrictions and the potential for severe climatic events – hurricanes, it is imperative that the spatial distribution of the disease be monitored within the state and within infected groves. This study investigates the spatial distribution in two commercial ‘Valencia’ citrus groves located within the quarantine zones mandated by the USDA. Trees were mapped for the presence or absence of CBS in groves. Positive trees were defined as having fruit with at least a single hard or shot-hole spot lesion. The hard or shot-hole spot lesion was chosen due to its ease of recognition as a symptom of CBS. Preliminary spatial analysis of disease intensity and clustering were analyzed in R using the binomial dispersion index (D), and Ripley’s K function (K). The incidence of CBS in Grove 1 and 2 were 7.1% and 2.6% respectively in the 2013-2014 citrus season. The binomial dispersion index (D), revealed clustering of symptomatic trees for both groves in all quadrant sizes tested. Monte Carlo simulations for the L function confirmed CBS aggregation in both groves. Monitoring is ongoing.

### **Sensitivity survey of *Alternaria* species to SDHI fungicides reveals regional and temporal resistance changes in pistachio orchards of California**

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In California, resistance of *Alternaria* spp. to succinate dehydrogenase inhibitor (SDHI) fungicides is common in pistachio orchards and results in difficulties in managing *Alternaria* late blight (ALB). The objective of this study was to monitor the development and spread of SDHI resistant *Alternaria* phenotypes and evaluate the cross-resistance among fluopyram (Fp), penthiopyrad (Pe), fluxapyroxad (Fd) and boscalid (Bo). In total, 798 isolates of *Alternaria* spp. were collected from 35 pistachio orchards where SDHI were used. *In vitro* assays were used to identify the frequency of SDHI resistant phenotypes and to compare the sensitivity distribution of isolates from previous years. The results revealed 85.8, 83, and 80% phenotypes in the current population resistant to Bo, Fd, and Pe, respectively. The Fp showed the smallest EC<sub>50</sub> value 16.74 µg/ml and the greatest frequency of sensitive phenotypes, at 45.8%. For the first time, highly resistant phenotypes for Fp were observed in California pistachio orchards, showing a moderate cross-resistance with Bo, Fd and Pe. Finally, the current population of *Alternaria* isolates (2015) showed sensitivity shifts toward resistance for Fd and Fp in comparison to those of the 2010 survey. Regional and temporal changes in the resistance of *Alternaria* spp. population will be discussed as well as the impact of future studies in order to delay SDHI resistance in California.

### **Teratosphaeria pseudoecalypti: An emerging pathogen with uncertain impact on *Eucalyptus* plantations**

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*Teratosphaeria pseudoecalypti* was first discovered in Queensland, Australia in 2010 causing a serious leaf blight disease known as Kirramyces Leaf Blight (KLB) on *Eucalyptus* commercial plantations. The pathogen was subsequently reported in 2014 in Argentina, Brazil and Uruguay, representing the first occurrence outside Australia. During 2015, a survey was conducted to assess prevalence, incidence and severity of KLB on *Eucalyptus camaldulensis* and *E. tereticornis* in Uruguay. A total of 80 plantations were visited, including these species and their hybrids. In each plantation, one transect of 20 trees in a row was scored for crown damage, based on percentage of defoliation and overall disease severity. The disease was found in all regions of the country. There was no clear pattern of distribution or severity of the disease with a distribution appearing to be generalized. Classification and regression tree analysis indicated that host species was the most important segregating factor based on severity, were *E. tereticornis* plantations showed a significantly lower severity than *E. camaldulensis* and their hybrids. KLB is widespread and serious on susceptible *Eucalyptus* spp., posing a threat to commercial plantation forestry in Uruguay. Future studies will concentrate on understanding the genetic diversity of a large collection of isolates that have emerged from this study and on selecting planting stock resistant to KLB.

### **The occurrence pattern of plant viruses and their vectors in pepper fields in Korea**

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Pepper viruses usually cause severe symptoms on pepper resulting in a significant loss of the yield. While many viruses have been reported to infect pepper, some of them, including Cucumber mosaic virus (CMV), Broad bean wilt virus 2 (BBWV2), Pepper mottle virus (PepMoV), Pepper mild mottle virus, Tomato spotted wilt virus (TSWV), Beet western yellows virus (BWYV), and Potato virus Y, have already caused epidemics in pepper in Korea. In this study, we performed nationwide surveys from May to October 2015 to examine the occurrence of pepper viruses. Interestingly, the incidence of TSWV was highest in May but decreased dramatically month by month, whereas the occurrence of BWYV and PepMoV was detected from July and increased gradually until October. In addition, CMV and BBWV2 are the most prevalent in July and August and their incidences were maintained at high levels until October. In our surveys, we also investigated the incidences of thrips and aphids. Interestingly, the rate of population of thrips was greatest in May but decreased dramatically from July. On the other hand, the population of aphids gradually increased from May to August and the rate of alate aphids in the population was increased in September in pepper fields. Taken together, our survey results on the occurrence of viruses and their insect vectors suggested that the incidence of the pepper viruses is highly correlated with the population dynamic and life-cycle of their insect vectors.

### **Field Survey of Tospoviruses Infecting Tomato in South Florida**

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Several viruses are known to infect tomato plants in south Florida, leading to serious losses to growers. *Tomato chlorotic spot virus* (TCSV), *Groundnut ringspot virus* (GRSV) and *Tomato spotted wilt virus* (TSWV), are three tospoviruses currently known to infect tomato in this area. These tospoviruses can cause losses in tomato ranging from a reduction in fruit quality to a complete destruction of the crop. TCSV was first detected from tomato plants in Homestead, FL in 2012, and it has caused significant losses to commercial tomato growers since the fall of 2014. During 2015-2016 growing season, a survey on tospoviruses infecting tomato in Homestead, Florida was conducted to determine the disease incidence and distribution of the tospoviruses in commercial tomato production fields. The percentage of tomato plants showing tospovirus-like symptoms in Homestead tomato fields ranged from 4 - 80% with an average of 35%. Two hundreds and seventy two tomato samples showing the symptoms were collected from over forty tomato fields. RNAs were extracted from the samples and tested by Reverse Transcription-Polymerase Chain Reaction (RT-PCR) using specific primers against TCSV, GRSV and TSWV to determine the incidence of both single and mixed infection of these viruses. RT-PCR results confirmed the infection of tomato plants with tospoviruses in Homestead. The results of the RT-PCR tests will be presented and more details will be discussed.

### **Spatial dynamics of a begomovirus disease in processing tomato in central Brazil**

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The begomovirus disease is one of the most important viral diseases in the tomato crop in Brazil. The objective of this work was to study the spatial aggregation of begomovirus disease in two central-pivot irrigated areas for processing tomatoes in central Brazil. A total of 24 plots (15 × 15 plants) was weekly evaluated by visual inspection for identification of begomovirus symptomatic plants (BSP). The spatial distribution of the disease was analyzed by: binominal index of dispersion, Taylor's power law, and ordinary run. *Tomato severe rugose virus* was the predominant begomovirus species in tomato. It was also detected in a few non-tomato plants. The spatial pattern of symptomatic plants was slightly aggregated. A relevant difference was observed between plots located at the center (PC) and at the edge (PE) of the pivots. The distribution of BSP in PC was more aggregated than in PE. It is most likely that at PE the primary spread is the major component of virus epidemics in the field. These results suggest that the distribution of BSP in PC and PE is ruled out by at least three different dissemination mechanisms: a random (primary spread), and two aggregation components, a real secondary spread, and a "false" secondary spread. However, it is clear that the primary spread is the most important component in begomovirus epidemics. Hence, management of begomovirus diseases focusing only on the target fields is certainly not enough for an efficient control.

### **Composition of leaf fibrous ring mediates *Diaphorina citri* preference of *Citrus sinensis* over *Citrus aurantium***

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*Candidatus Liberibacter asiaticus*, causal agent of citrus greening disease, is transmitted by *Diaphorina citri*, and together they pose the greatest risk to *Citrus* production worldwide. Currently research into host plant resistance to the vector and bacterial pathogen is ongoing, with the aim of developing resistant *Citrus* selections. Our objective was to test the influence of leaf anatomy on *D. citri* probing behaviors that regulate bacterial transmission. Leaf morphometrics might inhibit bacterial transmission, and thus could be candidates for resistance breeding. We performed an electropenetrographic study and histological examination of leaves of two *Citrus* species differently preferred by the psyllid, *C. sinensis* (var. Valencia) and *C. aurantium* (var. Sour orange). We quantified durations, frequencies, and order of probing behaviors associated with bacterial transmission, and correlated those behaviors with leaf anatomy. Young Sour Orange leaves were less preferred and exhibited a thicker and more complete fibrous ring than did same-aged Valencia leaves. This more developed barrier impacted probing behaviors such that *D. citri* took longer to reach the phloem and ingested from it for shorter durations. Delay and reduction of phloem associated behaviors influences the success of bacterial transmission by *D. citri*. Future resistant varieties could use fibrous ring morphometrics as an anatomical marker for resistance to pathogen transmission.

#### **Transmission of a 16SrIII-L phytoplasma by leafhoppers (*Scaphytopius marginineatus*) to cassava (*Manihot esculenta* Crantz) in Colombia**

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As much as 30% of cassava production in Colombia, is affected by cassava frogskin disease (CFSD). The disease is associated with a 16SrIII-L phytoplasma that is transmitted between plants through vegetative propagation or by sap-sucking insect vectors. We collected 250 insect samples in Palmira and Santander de Quilichao; analyzed them for the presence of phytoplasmas during 2009 and 2012–2014; and detected a 16SrIII-L phytoplasma. Greenhouse experiments with symptomatic plants showed that potential insect vectors for cassava were hemipterans, particularly the Cicadellidae and Cixiidae families, and include the cicadellid leafhopper *Scaphytopius marginineatus*. Colonies of this leafhopper were mass-reared on cassava ('SM 909-25') and bean ('ICA Pijao') plants. To determine the acquisition period, 50 individual insects were selected and exposed to phytoplasma-infected cassava plants for 2, 4, 6, and 8 days. The insects then underwent an additional 2 weeks' incubation period, after which surviving insects were allowed to infest healthy cassava plants over a 5-to-7-day inoculation period. Molecular studies of plant tissues, based on PCR amplification and DNA sequencing, confirmed both the acquisition (6 days after inoculation) and transmission (3 weeks) of a phytoplasma. Sequences KP75989, and KP759898-KP759902 were reported to GenBank. Previous studies indicated that potential infestations of cassava crops by leafhoppers can be controlled by organic neem oil spray.

#### **Cladosporium interactions with the introduced insect spotted wing drosophila (*Drosophila suzukii*) may alter raspberry fruit rot epidemiology**

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In fruit crops, the interaction between frugivorous insects and fungi can alter pest and disease epidemiology, reducing marketable crop yields. In 2015 field surveys of fall red raspberries in Maryland, *Cladosporium* was recovered from fruit-rot affected berries at three farms, and 30% of *Cladosporium*-infested berries were co-infested with SWD larvae. Analyses of larval frass microbes recovered *Cladosporium* from 45% of larvae, with 3.3 colony forming units/ larvae on average. Sequence analysis identified both berry and larval frass isolates as either *Cladosporium cladosporioides* or *C. pseudocladosporioides*. All three tested isolates of each species were pathogenic on raspberries in detached berry assays, and one tested isolate could infect both ripe and unripe fruit in attached-berry assays. Disease incidence was significantly enhanced ( $P < 0.05$ ) both when inoculated detached berries were mechanically wounded and when naturally infested attached berries were exposed to lab-reared SWD adults (ovipositor wounds), compared to unwounded controls. Many endophytic *Cladosporium* species are entomopathogens, so species causing fruit rot may be deleterious to SWD fitness; alternately, this may represent a commensal or mutually beneficial interaction. Overall, these results are among the first to suggest that SWD may interact with *Cladosporium* to significantly alter pre- and post-harvest fruit rot epidemiology.

#### **The ability of *Olpidium brassicae* to vector blueberry mosaic virus in southern highbush blueberries**

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Blueberry mosaic associated Virus (BIMaV) is a recently described ophiovirus that is of concern in blueberry propagation systems. Members of the fungal genus *Olpidium* commonly found in plant roots, have the ability to move in these production systems using flagella, and possess the ability to vector Ophioviruses. In 2015, BIMaV affected blueberry plants were identified in a propagation nursery and a series of trap plants were utilized to determine potential vectors. Trap plant experiments were conducted with varying soil treatments (n=5), and hosts (carrot, lettuce, broccoli, cucumber) to determine the presence of *Olpidium* spp. *Olpidium* was observed in only lettuce trap plants (average of 6 resting spores/cm of root) and absent in the remaining trap plants. The ability of *Olpidium* species to vector BIMaV was tested through a series of concurrent inoculation experiments consisting of inoculated groups and control groups, treated with lettuce roots containing *Olpidium* resting spores or water, respectively. Each series of inoculation experiments varied in exposure time (10, 8, and 6 months) to root inoculum containing *Olpidium* resting spores and water, respectively. Transmission experiments showed *Olpidium* to be readily observed in the roots of inoculated southern highbush blueberry. By determining the ability of *Olpidium* spp. to vector BIMaV will aid in the management of blueberry propagation and increase our understanding of the basic biology of BIMaV.

#### **Olive Escudete, caused by *Botryosphaeria dothidea*, as Result of the Interaction fly-mosquito-fungus**

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The Dalmatian disease or escudete, caused by *Botryosphaeria dothidea*, is the most important disease of table olive fruit. The typical escudete (small shield) symptom is a sunken, necrotic, and circular ( $\approx 8$ mm) lesion that appears in green fruits. The olive fly (*Bactrocera oleae*) and mosquito (*Prolasioptera berlesiana*) whose larvae prey on fly eggs, are disputed as vectors of the pathogen. Here, we study the interaction among these three agents: fly-mosquito-fungus. In the field, we periodically sampled for the presence of the mosquito in fruits, which were naturally punctured by the olive fly or artificially by a needle for three seasons. Under lab conditions, the presence of the fungus in the mosquito and the fly was studied by microscopy, isolation on media, and DNA sequencing. According to our results, the mosquito is attracted by ovipositor punctures on the olive surface made by the olive fly. When the mosquito deposits its own egg adjacent to the fly egg, it also inoculates the fungus in the puncture. This allows the mosquito larvae to feed on *B. dothidea* mycelium when the fly egg is absent; a need that arises due to frequent false punctures (no oviposition) made by the fly to avoid predation by the mosquito larvae. Microscopic studies show that the mosquito female has a special structure (mycangia) to carry *B. dothidea* spores in the last abdominal segments close to the ovipositor, which suggests a mutualistic association between *B. dothidea* and *P. berlesiana*.

#### **First report of laurel wilt on sassafras in Arkansas**

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Laurel wilt, caused by the fungus *Raffaelea lauricola* T.C. Harrin., Aghayeva & Fraedrich, is a lethal disease of redbay (*Persea borbonia* (L.) Spreng.), sassafras (*Sassafras albidum* (Nutt.) Nees), and other species in the laurel family (Lauraceae). The fungus is transmitted by an exotic ambrosia beetle (*Xyleborus glabratus* Eichh.) native to Asia introduced into the United States in 2002. It was first reported at Port Wentworth, Georgia and rapidly spread across the south causing extensive redbay mortality. On December 2, 2015, visit to a landowner in Bradley County near Warren, Arkansas (33.64511°N, 92.05134°W) led to the first reported mortality of sassafras caused by laurel wilt in Arkansas. Moderate degrees of staining and beetle galleries were found from symptomatic trees at this location. Suspect beetles collected from sapwood of affected trees were later identified as *X. glabratus*. The presence of *R. lauricola* was confirmed by morphological characterization and molecular identification of cultures obtained from stained sapwood tissues. This is the first report of laurel wilt on sassafras or any other Lauraceae species in Arkansas. In 2014, laurel wilt was reported on sassafras in northern Louisiana (83 miles southwest). This new report in Arkansas may indicate northward movement of the disease into natural habitat of sassafras. However, the lack of redbay (a more attractive host) in this region may slow the northward spread of laurel wilt in Arkansas.

#### **Determination of vector population trends, species composition, and the role they play in the barley yellow dwarf complex in Northeastern Kansas**

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Barley yellow dwarf is a complex disease that includes grass hosts, aphid vectors, and yellow dwarf viruses (YDVs) in the family *Luteoviridae*. BYD is responsible for crop losses worldwide, and is an especially important disease of KS wheat. The goals of this project were to characterize aphid vector species composition in native grasses and crops, and to document associated YDVs in NE KS. Aphid populations were monitored during fall, spring, and summer using yellow sticky cards that were placed in fields of sorghum, corn, wheat, and big bluestem, and aphids were identified via morphological features. *Rhopalosiphum padi* was found to be the most prevalent aphid species. Other species of importance were *Sitobion avenae*, *R. rufiabdominalis*, and *Schizaphis graminum*. Aphid species overlapped between crops and native grasses, supporting the hypothesis that perennial grasses can serve as reservoirs for YDVs. Multiplex RT-PCR was used for virus detection in plants and aphids, and both tested positive for several YDVs. We found barley yellow dwarf virus (BYDV)-PAS to be the virus species most common in both aphids and plants, followed by BYDV-PAV and BYDV-RPV. Aphids and plants were either singly- or co-infected with YDV species. Ultimately, this will lead to determination of the most efficient vectors of YDVs, their association with YDV-species composition in croplands, and the temporal influx of viruliferous aphid populations across agroecosystems in KS.

#### **Variation in transmission of Tomato spotted wilt virus (TSWV) from Thrips tabaci is not solely influenced by virus titer within individual thrips**

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*Thrips tabaci*, a vector of TSWV, has been shown to vary greatly in the ability to transmit different isolates of TSWV. The objective of this study is to determine if the virus titer in individual *T. tabaci* influences the variation in transmission of TSWV from established *T. tabaci* isofemale lines collected from several locations in North Carolina. The isofemale lines were previously shown to differ in their ability to transmit various TSWV isolates collected from the same series of locations as the thrips. The virus-thrips line combinations were grouped in sympatric and allopatric pairings and the groups classified as low, moderate, or high transmitters based on the percent transmission of TSWV to *Emilia sonchifolia* leaf discs. Quantitative real-time PCR was done on *T. tabaci* from 12 selected virus-thrips line combinations to quantify virus titer in transmitting and nontransmitting individuals to determine if the variation in transmission was correlated with virus titer. The results show that variation in transmission efficiency of the different virus-thrips pairings are not explained by differences in virus titer within the thrips. This is in contrast to findings with other TSWV thrips vectors where virus titer was directly correlated with transmission efficiency. This suggests that the differences in transmission among these thrips-virus isolate pairings may be determined by the genetics of both the virus and the thrips, and their interaction.

#### **Turnip aphid preference for turnip plants infected with different strains of Cauliflower mosaic virus**

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*Cauliflower mosaic virus* (CaMV) is transmitted in a semi-persistent manner by several species of aphids. In this study, we investigated whether the turnip aphid (*Lipaphis erysimi*) exhibits a preference for healthy turnip plants (*Brassica rapa* L. var. *rapa*) or plants infected with CaMV. Two strains of CaMV, W260 and NY8153, as well as one recombinant isolate, H12, were used in this study. Preference assays were conducted with detached leaves in petri dishes or with whole plants. When given a choice between infected or healthy plants, aphids chose W260-infected and H12-infected plants significantly more often, regardless of the test. Interestingly, aphids did not exhibit a preference for turnips infected with CaMV strain NY8153 over healthy plants. The recombinant isolate H12 was chosen because it does not elicit symptoms in turnips; the aphid's preference of H12-infected plants over healthy turnips indicated that aphids were not attracted to plants based on differences in color or a pattern associated with disease. We are now focused on identifying differences in volatile profiles between healthy, W260- and NY8153-infected turnips that might influence the preference of an aphid for a specific host. These results suggest that CaMV infection affects the aphid's choice of host and this in turn may have implications for the spread of different strains of CaMV.

#### **Aphid-mediated potyvirus transmission: Host plant chemistry, vector biology and component interactions**

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Potyviruses pose a significant threat to cucurbit production in Georgia and in the Southeastern United States. Aphids transmit these viruses in a non-circulative manner, causing extensive yield losses worth millions of dollars annually. The present virus and aphid management strategy involves the use of insecticides and transgenic cucurbit cultivars. However, none of these approaches have proven to be sustainable, especially due to the development of vector resistance to insecticides, and the narrow spectrum of virus resistance in transgenic cultivars. It is therefore critical to explore the novel avenues for reducing vector populations and viral incidences. To attain this, we have undertaken a holistic approach that explores the fundamental mechanisms underlying aphid-potyvirus interactions in cucurbit systems. Through an array of lab and field experiments, we have been studying the virus transmission, component interactions in the pathosystem and their impacts on vector behavior, fitness and transcriptome. The impact of these results in the context of virus epidemics and management will be discussed.

#### **Transovarial and Sexual Transmission of Tomato yellow leaf curl virus in Whiteflies**

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*Tomato yellow leaf curl virus* (TYLCV) is whitefly-transmitted Geminivirus that causes yield losses in tomato crops. The virus is persistently transmitted by the sweetpotato whitefly, *Bemisia tabaci*. The virus is considered non-propagative and non-transmissible transovarially or through mating. Feeding on infected plant tissue is the only method of acquisition according to most of the literature. However, a few recent studies from Israel demonstrate transovarial and sexual transmission of TYLCV as well as propagation within the whitefly. We have tested to see if transovarial and sexual transmission occur with the whiteflies and TYLCV present in Georgia using both molecular methods and plant transmission experiments. We found a very low rate of transovarial and sexual transmission suggesting negligible impact on virus epidemics. COI sequences from our local *Bemisia tabaci* and full-length TYLCV

genomes have been compared with sequences from Israel available on GenBank. Our *B. tabaci* COI haplotype is present in Israel, but differences in the TYLCV genome were found at the amino acid level. These differences could potentially explain the differences seen in insect-virus interactions.

#### **Molecular interactions between citrus tristeza virus and its aphid vector, *Toxoptera citricida***

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Citrus tristeza virus is transmitted by several aphid species, including *Aphis gossypii*, *Aphis spiraecola*, *Toxoptera aurantii*, and *Toxoptera citricida*. Considerable variations in the efficacy of transmission both among different vector species, and among CTV isolates are recorded. *T. citricida*, the brown citrus aphid, is the most efficient and specialized vector. It has a host range limited primarily to rutaceous species and transmits most CTV isolates at higher rates than other aphids. We demonstrated that CTV binds specifically to the lining of the cibarium of the aphid using fluorescently-labelled virions. *In vitro* competitive-binding assays between fluorescent virions and free viral proteins determined that the minor coat protein and two heat-shock-like proteins (p61 and p65) are involved in vector interaction. Treating the dissected mouthparts with proteases prior to the binding assay did not affect the binding of CTV virions while, chitinase treatment reduced the CTV binding to foregut. These findings suggest that CTV binds to sugar moieties of the cuticular surface of the aphid cibarium and not to embedded proteins. Additionally, the specific binding involves the concerted activity of three virally-encoded proteins.

#### **A strategy to study population variation of *Xanthomonas axonopodis* pv. *manihotis* using a new set of multiple-locus variable number tandem repeats**

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Multiple-Locus Variable Number Tandem Repeat Analysis (MLVA) is being used to analyze genetic diversity in phytopathogenic microorganisms. One of the most important pathogens affecting Cassava crops is *Xanthomonas axonopodis* pv. *manihotis* (*Xam*) as its infection relates to high losses in crop productivity. Several studies have reported that *Xam* populations change over time. It is important to assess population diversity in this pathogen as one of the basis to develop cassava resistant varieties. Studies to typify *Xam* strains performed by our group showed that using Variable Number of Tandem Repeats (VNTRs) have advantages over Amplified Fragment Length Polymorphisms (AFLPs), such as low costs and high discriminatory power using small sets of markers. Consequently, in this study we standardized and tested a new scheme of VNTR loci to analyze *Xam* populations. First, by analyzing 12 of 65 previously sequenced genomes of *Xam*, which represent the worldwide variability, we selected 16 VNTR loci. We standardized four multiplex PCR to amplify four microsatellite loci in each multiplex. Finally, the 16-VNTR scheme was used to assess population diversity of *Xam* strains collected in four Colombian Caribbean-region localities from 2008 to 2010, 2013 and 2014. Our results show that most of *Xam* genetic diversity is found within populations, and that the 16-VNTR scheme is a useful tool to discriminate *Xam* populations up to a local scale.

#### **Diversity of *Aspergillus flavus* on important agricultural crops produced in Bangladesh**

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The hot, humid climate of Bangladesh is ideal for aflatoxin producing fungi. As a result, rice, maize, peanut, wheat and other crops grown in Bangladesh may become infected by these fungi and contaminated with dangerous concentrations of aflatoxins. Aflatoxins are toxic fungal metabolites produced by *A. flavus*, *A. parasiticus*, and closely related species. Aflatoxins cause increased risk of liver cancer, stunting, immune suppression, and in cases of acute exposure, liver necrosis and death. However, frequencies of aflatoxins and diversity of aflatoxin-producing fungi in crops of Bangladesh are poorly understood. To assess the magnitude of aflatoxin contamination and diversity of *A. flavus* in crops produced in Bangladesh, maize, rice, wheat and peanut samples were collected from 12 districts covering the major agro-ecological regions of Bangladesh. Aflatoxins were quantified in crop samples using immunochromatographic assays. Variable levels of aflatoxins were detected across the samples with a maximum concentration of 122.8 µg/kg total aflatoxins. Fungi associated with the maize and belonging to the L-strain morphotype were genotyped using 16 SSR markers. Genetic diversity of the L morphotype was high with 5 to 24 alleles per SSR locus, and 46 haplotypes among 50 isolates. Atoxicogenic strains of *A. flavus* of potential value in aflatoxin prevention and native to Bangladesh were characterized.

#### **The genetic structure, virulence, and fungicide sensitivity of *Fusarium fujikuroi* in Taiwan**

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*Fusarium fujikuroi* is the causal agent of bakanae disease, a common rice disease causing abnormal growth of seedlings and poorly filled grains. In Taiwan, the disease has long been well managed, but a disease outbreak was found in eastern Taiwan in 2009. To better understand the population genetics of *F. fujikuroi*, 16 polymorphic simple sequence repeat markers were newly developed and used to analyze 637 *F. fujikuroi* isolates collected in 14 rice-growing areas around Taiwan from 1996 to 2013. The isolates were classified into four highly differentiated clusters by Bayesian clustering analysis. The highly diversified vegetative compatibility groups and good numbers of *MATI-1* and *MATI-2* isolates sampled from all around Taiwan supported sexual reproduction. However, the biased mating type ratios, reduced effective population numbers, and linkage disequilibrium suggest nonrandom mating between individuals. Analysis of isolation by distance revealed a significant relationship between spatial distance and genetic similarity, which implies a geographical restricted gene flow and low dissemination ability of *F. fujikuroi*. Evaluation of 24 representative isolates on 8 rice varieties revealed differential levels of virulence, however no clear pattern of specific variety x isolate interaction was observed. Investigations of 60 representative isolates from early and recent years indicate that *F. fujikuroi* in Taiwan has developed increased resistance to prochloraz.

#### **Exploring genetic diversity in *Macrophomina phaseolina*, the causal agent of charcoal rot on soybean**

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*Macrophomina phaseolina* is a soil and seed-borne fungal pathogen infecting more than 500 plant species and causing charcoal rot disease on soybean (*Glycine max*). Recently, a set of *Macrophomina* isolates collected from three plant species in Senegal, morphologically similar and phylogenetically closely related to *M. phaseolina*, were described as a new species, *M. pseudophaseolina*. However, occurrence of *M. pseudophaseolina* is unknown outside of Senegal. In this study, 36 putative *M. phaseolina* isolates, collected from soybean grown in the northern and the southern US were identified to the species level. ITS, EF-1 $\alpha$  and ACT loci were sequenced and analyzed. Additional sequences were obtained from GenBank. The Incongruence Length Difference (ILD) test was not significant (p=0.95) and supported a decision to combine the sequences. Phylogenetic relationships of the isolates were explored using Maximum Parsimony (MP), Neighbor-Joining (NJ), Maximum Likelihood (ML) and Bayesian Inference (BI). The MP, ML, NJ and BI

phylogenies resulted in two separate clades corresponding to *M. phaseolina* and *M. pseudophaseolina* and placed all 36 isolates from soybeans in the U.S. in *M. phaseolina*. The Shimodaira-Hasegawa test indicates the ML tree as the best, but there were not significant differences among any of them. The ML tree reconstructed the *M. phaseolina* and the *M. pseudophaseolina* clades with a bootstrap support value of 100%.

#### Population structure of the wheat pathogen *Zymoseptoria tritici* in France at different scales

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*Zymoseptoria tritici* is currently the most damaging disease on wheat crops in Europe, especially in France, where it causes each year 1.7 t/ha yield loss in average. In order to better understand its genetic features, we characterized several *Z. tritici* populations (more than 1500 isolates), isolated in France over a ten-year period (2005-2015) and at different spatial scales (whole country, Nord-Pas de Calais region, field, 30 cm<sup>2</sup> square, plant, leaf and lesion) using SSR markers and mating type idiomorphs. Results revealed high and similar levels of genetic and genotypic diversities at the country, region and field scales, but the diversity values gradually decreased from the field to finer scales such as plant, leaf and lesion. Bayesian and non-Bayesian statistical analyses revealed significant structuration of the global French population into three genetic clusters, and the Nord-Pas de Calais population into three sub-clusters, all distributed according to their geographical origin. However, no differentiation was found at finer scales. Both mating types were found at equal proportions at the country, region and field scales and co-occurred at all other investigated scales. This study highlighted for *Z. tritici* in France a high potential for sexual reproduction and genetic diversification, as well as a structuration of its populations at largest scales, agreeing with its high fitness degree and its ability of adaptation to local agro-climatic conditions.

#### Stripe rust epidemics of wheat and barley and races of *Puccinia striiformis* identified in the United States in 2015

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In 2015, stripe rust of wheat, caused by *Puccinia striiformis* f. sp. *tritici* (*Pst*), occurred throughout the United States and caused severe yield losses in the Great Plains. Stripe rust of barley, caused by *P. striiformis* f. sp. *hordei* (*Psh*), occurred in California, Montana, Oregon, Utah, and Washington. For monitoring virulence changes of the pathogens, stripe rust samples collected from 21 states were tested on 18 wheat and 12 barley differentials to identify *Pst* and *Psh* races, respectively. From 32 *Psh* isolates, three previously existing races were detected, of which PSH-48 (virulent only on Topper among the 12 barley differentials) and PSH-33 (virulent on differentials Topper and Abed Binder 12) were the major races with frequencies of 53% and 54%, respectively, similar to 2014. From 310 *Pst* isolates, 32 races were detected including 5 new races (PSTv-140, PSTv-141, PSTv-142, PSTv-143, and PSTv-144). The top five frequent *Pst* races were PSTv-37 (36%), PSTv-52 (33%), PSTv-18 (5%), PSTv-53 (4%), and PSTv-48 (3%). PSTv-37 and PSTv-52 were detected in every epidemiological regions, while PSTv-18, PSTv-53, and PSTv-48 were detected only in the western U.S. High virulence frequencies (>50%) were detected for *Yr6*, *Yr7*, *Yr8*, *Yr9*, *Yr17*, *Yr27*, *Yr43*, *Yr44*, and *YrExp2*; moderate (10-50%) for *Yr1*, *YrTr1*, and *YrTye*; low (<10%) for *Yr10*, *Yr24*, *Yr32*, and *YrSP*; and none for *Yr5* and *Yr15*. The information is useful for breeding for resistance and management of stripe rust.

#### Preliminary screening of genetic markers for population analyses of *Magnaporthe oryzae* Triticum pathotype

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Wheat blast, caused by *Magnaporthe oryzae* Triticum pathotype (MoT), is a devastating disease of wheat in South America; primarily in Brazil, Bolivia and Paraguay, and recently in a small area of northeast Argentina. It is a potential threat to wheat production worldwide with implications for food security. Very little is known about the population biology of the MoT. Therefore, understanding the population biology of wheat blast is important. Screening of current populations from Bolivia, Brazil, and Paraguay was performed using 30 inter-simple sequence repeat (ISSR) and retrotransposon microsatellite amplified polymorphism (REMAP) primers. Genes for MLST were also screened using primer sets targeting conserved genes and genes involved in different cellular functions. Combined phylogenetic analyses using ISSR and REMAP loci was able to differentiate populations from the three different regions based on 151 total loci, including 119 polymorphic and 32 monomorphic loci; showed 78.80% of percent polymorphism. The MLST based on genes involved in cellular function was found to be informative for the population genetics study. The screening of more markers for the population study is underway. This study will provide valuable information about genetic variation within and among current MoT populations of value for disease management and for the development of reliable and accurate detection tools for this emerging disease.

#### Population structure and genetic diversity of *Fusicladium effusum* in the USA

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Scab (*Fusicladium effusum*) is the most destructive disease of pecan in the Southeast US. Infection is thought to occur solely through asexually produced conidia. To explore the population structure and genetic diversity of *F. effusum*, populations were hierarchically sampled from 11 orchards in Alabama, Florida, Georgia, Illinois, Kansas, Louisiana, Mississippi, Oklahoma and Texas. A total of 734 isolates was collected from up to 20 leaves from each of 3 to 6 trees in each orchard. Isolates were screened against 30 microsatellite markers. Polymorphic loci per population were  $\geq 96.7\%$ . Populations were genetically diverse (Nei's measure of genetic diversity = 0.505-0.692). An analysis of molecular variance showed 81% of variation occurred in the populations of *F. effusum* within trees, 3% in populations between trees within orchards, and 16% between orchards. Population differentiation was low ( $G_{ST} = 0.17$ ) and gene flow relatively high ( $N_m = 2.5$ ). However, there was some association between population genetic and log physical distance ( $r = 0.471$ ,  $P = 0.0003$ ). Ten of the 11 populations showed evidence of gametic disequilibrium ( $r^2_d = 0.024-0.057$ ), which might be explained by a predominance of within-season asexual reproduction, or possibly by founder effects or migration rates. The results show that *F. effusum* is a genetically diverse pathogen, with the degree and distribution of genetic diversity often associated with sexual recombination.

#### Spatial and temporal structure of *Sclerotinia sclerotiorum* in New York State

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*Sclerotinia sclerotiorum* causes white mold on many vegetable and field crops in New York State, but the population structure has not been thoroughly studied. To assess spatial and temporal variation of *S. sclerotiorum* in New York State, 200 isolates were sampled from 10 bean fields in 2014, and 36 isolates were obtained from a historical collection (sampled from various hosts between 1982 and 2008). Isolates were genotyped using eight microsatellite loci. Mycelial compatibility group (MCG), in vitro growth rate, and aggressiveness on snap bean plants were also assessed for 22 selected isolates. Twenty-eight unique multilocus genotypes (MLGs) were identified (24 in 2014). One MLG represented 44 and 47 percent of isolates sampled in 2014 and prior to 2014, respectively. In 2014, genotypic diversity was low (3 to 8 MLGs per field) and Nei's genetic diversity varied among fields (0.42 to 0.90). Analysis of molecular variance indicated no significant spatial structure within or between fields, and the index of association differed significantly from zero in all fields. Isolates of the same MCG almost always shared the same MLG. Growth rate and aggressiveness varied slightly but

significantly among some isolates, but no significant association was found between the two measurements. Thus, the *S. sclerotiorum* population in New York State is highly clonal, and a small number of representative isolates would likely be sufficient to test new disease management strategies.

#### **Sub-lethal fungicides induce microsatellite mutation in *Sclerotinia sclerotiorum***

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Stress-induced mutations from sub-lethal fungicide exposure may be an important source of genetic variation for clonal plant pathogens. We used *Sclerotinia sclerotiorum* to assess the role of sub-lethal fungicide exposure in mutation at microsatellite loci. Nine sensitive isolates were exposed to long-term, sub-lethal doses of fungicides with different modes of action: boscalid, iprodione, thiophanate methyl, azoxystrobin and pyraclostrobin. Fungicides were plated in a logarithmic gradient and each isolate sub-cultured from the 50-100% inhibition zone for 12 consecutive generations and experiment repeated. Isolates at the first and twelfth generation from exposed and non-exposed lines were genotyped at six microsatellite loci. Control isolates showed no difference after 12 generations. Five of nine and two of nine fungicide-exposed isolates showed microsatellite mutations in both experiments. One locus did not mutate; all others range from 1 to 34 mutations per locus, with an average of 4.58 stepwise mutations. Most mutations were less than 20 bp, with one 80 bp mutation. The average mutation rate per locus among fungicide-exposed isolates ranged from 4-29 fold greater than the expected mutation rate of  $1.3 \times 10^{-5}$  estimated for microsatellites in *Saccharomyces*. All fungicides resulted in mutations, with iprodione- and azoxystrobin-exposed isolates showing the highest frequency. Results suggest long-term sub-lethal fungicide exposure increase mutation rates.

#### **Molecular characterization and genetic diversity of *Mycosphaerella fijiensis* in Costa Rica using sequence based nuclear markers**

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*Mycosphaerella fijiensis* is the causal agent of black sigatoka, a devastating disease of banana that can cause 20-80% yield loss in the absence of fungicides. *M. fijiensis* first appeared in Honduras in 1972 and spread to the rest of the western hemisphere, including major banana production regions such as Costa Rica. In order to characterize the genetic diversity of *M. fijiensis* in Costa Rica, one hundred and eighteen isolates, collected from Costa Rica and Honduras between 2010 and 2014, were examined using multilocus genotyping of six loci and compared to a previously published global dataset. A total of 13 haplotypes were identified from concatenated sequences of Honduran and Costa Rican populations, six of which were unique. Outside of the Americas, Costa Rican and Honduran populations shared haplotype diversity with populations in both Oceania and Southeast Asia for all loci, in particular Australia, Futuna, Tonga, and Papua New Guinea, the latter of which has been proposed as the center of origin for both banana and *M. fijiensis*. The genetic similarity to both regions suggests *M. fijiensis* may have been introduced into Honduras and Costa Rica multiple times. Given a relatively recent introduction, subsequent admixture events are expected to increase diversity of Costa Rican populations over time.

#### **Genetic and phenotypic diversity of *Puccinia kuehnii* of sugarcane from Brazil**

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Resistant genotypes are the most employed method to control orange rust of sugarcane (*Puccinia kuehnii*). This approach has been adopted in Brazil; however, recent outbreaks on previously unreported varieties show that the efficacy of this method is dependent and requires better knowledge of pathogen diversity. This can be achieved by applying genetic and phenotypic tools. Therefore, the present work aimed to examine the genetic distance and pathotype variability among isolates of *P. kuehnii* from São Paulo, the most important growing state of the Country. Thirty six rust isolates were genotyped by eight SSR markers and their genetic distances verified by the UPGMA clustering analysis using the software NTSYSpc v.2.2. Phenotypic data employed 22 isolates inoculated on five sugarcane varieties and evaluated at 21 days by examining percent diseased leaf area with Assess 2.0 and rating resistant/susceptible based on Santos et al. (2004). Moreover, an assay of disease progress over time was carried out with three isolates against five sugarcane cultivars examining disease score (Tai et al., 1981) at days 21, 28, 35 and 42. Our results showed two genetically distinct subpopulations of the pathogen with 79% of diversity, phenotypic data identified nine physiological races. In addition, disease progress over time showed that one cultivar considered resistant at day 21 became susceptible at day 42. Part of MSc dissertation by the second author at PPGVBA, CAPES Scholarship.

#### **Short-term Host Selection Pressure Has Little Effect on the Evolution of *Verticillium dahliae***

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Understanding pathogen evolution over time is vital for breeding and deployment of host resistance. To study this in the context of a soilborne pathogen, within-field population structure from three major hosts (spinach, tomato and lettuce) and the potential of host-directed evolution of *V. dahliae* race 1 were determined. A total of 408 representing 192, 44, and 172 isolates from spinach, tomato and lettuce, respectively, were collected from WA and CA. An additional 411 isolates were recovered over five years from an experimental screening plot infested with a race 1 strain in Salinas, CA. Isolates were genotyped using 13 microsatellite loci, race and mating type-specific primers. Results indicated no significant variation among the within-field populations from the three hosts. Isolates recovered from the screening plot over five years revealed no demonstrable changes in the race, species composition and mating type structure, except for six SSR locus variants. Replicated virulence assays of these six variants with the wild type race 1 strain on susceptible and resistant lettuce differentials suggested no significant virulence differences between them. This suggests that the deployed host resistance will remain stable against the native *V. dahliae* population, unless novel pathogen genotypes are introduced into the system.

#### **Population structure of *Sclerotinia subarctica* in England, Scotland and Norway**

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*Sclerotinia subarctica* is closely related to *S. sclerotiorum* which is a major pathogen of a wide range of dicotyledonous crop plants in many countries across the world. In contrast to *S. sclerotiorum*, *S. subarctica* is little studied as it was only identified relatively recently. However, preliminary evidence suggests that it is usually found sympatrically with *S. sclerotiorum* on the same host plants, but may be confined geographically to more northern latitudes. Using molecular approaches, we established that *S. subarctica* was rarely found in crop plants or wild buttercup in England (4.7% of *Sclerotinia* sp. isolates) and was more prevalent in Scotland (18.3%) and Norway (48.0%). Microsatellite data analysis of 157 *S. subarctica* isolates revealed a multiclinal population structure (75 haplotypes) with one or a few haplotypes more common than the rest. Eight haplotypes were shared between populations from Scotland and Norway while none were shared with England. *S. subarctica* isolates from England were also less diverse and confined to one location suggesting geographic isolation. Preliminary data on the effect of temperature on mycelial growth and sclerotial germination *S. subarctica* is discussed in relation to the pathogen's distribution.

#### **Tools for analysis of clonal population genetic data in R**

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Knowledge of the population dynamics and evolution of plant pathogens allows inferences on evolutionary processes involved in their adaptation to hosts, pesticides, and other environmental pressures. These microbial pathogens often require different tools for analysis due to the fact that they can be clonal or partially clonal. With the advent of high-throughput sequencing technologies, obtaining genome-wide data has become easier and cheaper than ever before with techniques such as genotyping-by-sequencing. In 2013, we created the widely used R package poppr for analysis of clonal populations. In 2015, we published several additional extensions to poppr for use with genomic data including the ability to define clones based on a genetic distance threshold, minimum spanning networks with reticulation, and sliding window analysis of the index of association. We present here an overview of poppr as it pertains to traditional and high-throughput population genetic data with select applications.

#### **Is host specificity influencing the population structure of *Phytophthora* spp. collected from potato and tree tomato in central and southern Colombia?**

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Late blight, caused by *Phytophthora infestans*, has been a major threat to global food security. In Colombia, late blight is considered one of the most limiting diseases on potato and tomato production. Additionally to potato and tomato crops, the pathogen has been associated with outbreaks on semi-domesticated plants such as tree tomato (*Solanum betaceum*) and other Solanaceous plants. Recently, a new *Phytophthora* species has been described infecting tree tomato crops in Southern Colombia (State of Nariño). Nonetheless, the distribution range of this new emerging pathogen in the country is unknown. Thus, the aim of this study was to investigate: i) if populations of *Phytophthora* associated to tree tomato in the central region of Colombia (States of Antioquia and Cundinamarca), the largest potato producing regions in the country, are genetically different from populations found on potato crops in this region; ii) if host preference exists in populations of *Phytophthora* associated to tree tomato and those associated to potato crops in this region; and iii) if populations of *Phytophthora* associated to tree tomato in the central region of Colombia are genetically distinct from those found in the southern region of the country. Preliminary results indicate that all isolates are of the A1 mating type and that host preference seems to exist between populations of *Phytophthora* associated to tree tomato and those associated to potato crops in the central regions of Colombia.

#### **Thirty-three years of lettuce downy mildew and counting: A phenotypic and genotypic narrative**

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Downy mildew, caused by *Bremia lactucae*, is the number one foliar lettuce disease in California and worldwide. This heterothallic oomycete pathogen is highly variable and can rapidly change to overcome resistant cultivars. We are characterizing the pathogen population to determine which sources of wild germplasm will provide effective resistance in California that minimizes dependency on chemical protectants and aids organic farmers. We have been monitoring and archiving isolates of *B. lactucae* in California since 1982, mostly on an opportunistic basis. In December 2013, we began monthly surveys to provide a more comprehensive data set on pathogen variability. We report the patterns in virulence phenotypes observed in the Californian *B. lactucae* population over the past 33 years and an increase in the B<sub>1</sub> mating type. The Californian population has predominantly been the B<sub>2</sub> mating type and reproduces asexually. The occurrence of the B<sub>1</sub> mating type indicates the potential for sexual reproduction with prevalent B<sub>2</sub> isolates. Sexual progeny between recent B<sub>1</sub> and B<sub>2</sub> isolates are fit and highly variable and may explain the recent rise in novel virulence phenotypes. This finding necessitates a change in strategy to achieve more durable disease resistance. We have also sequenced the genomes of representative isolates collected throughout the three decades as a tool for understanding variation of *B. lactucae* in California and the world.

#### **Population structure of *Pseudoperonospora cubensis* isolates in Michigan and Ontario, Canada**

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*Pseudoperonospora cubensis* is a highly destructive obligate oomycete pathogen that incites downy mildew disease in cucurbits worldwide. The pathogen populations in Michigan and Ontario are primarily affected by the movement of sporangia annually from the southern U.S. and Mexico or northern greenhouses where the pathogen can overwinter. The objective of our research was to monitor the population structure change over time in Michigan and Ontario, Canada. Sporangia were collected from cucurbit downy mildew (CMD) lesions using leaves of field grown cucurbits from 2007 to 2010, and sporangia collected in 2015. Population structure and genetic diversity were characterized using three marker types: (1) Selected genomic regions from three mitochondrial and nine nuclear genes previously used in oomycete genetic diversity and phylogenetic studies; (2) Selection from 942 simple sequencing repeat (SSR) markers derived from *P. cubensis* reference genome that were inside or partially overlapping with the annotated transcripts; (3) Genomic regions containing higher frequency of host and/or species specific single nucleotide polymorphisms (SNPs) adapted from previous studies. This research provides insight into changes of CMD populations in Michigan and Ontario over time which may contribute to overall management strategies.

#### **A shift in the population of *Phytophthora infestans* on Egyptian potato crops**

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Potato is an economically important crop in Egypt that is cultivated over three growing seasons, summer, fall (Nili) and winter. The winter crop that is grown from September to the end of February is particularly prone to late blight caused by *Phytophthora infestans* due to weather conditions that are conducive from the start of November and many crops suffer severe yield losses. We obtained 145 purified *P. infestans* isolates from the 2013 and 2014 winter crops which were genotyped using 12 SSR markers to generate data that would inform disease management. In contrast to previous work (3-year period 2010-12) in which the 13\_A2 lineage was found, all isolates belonged to the 23\_A1 lineage. There was no evidence for the existence of the A2 mating type or 13\_A2 lineage even in the destroyed field crops of some cultivars (Cara, Bellini and Valor) that had been reported as resistant to 23\_A1. This suggests that the previously reported virulence profile of 23\_A1 has changed. Unlike 13\_A2 the 23\_A1 genotype is reported as sensitive to intermediate in response to metalaxyl. Polymorphisms in mainly 3 loci (SSR3, G11 and D13) discriminated 14 sub-clonal lineages of 23\_A1 in Egypt suggesting the population has been present in Egypt for several years. The data have entered into the Euroblight database to allow temporal and spatial genetic diversity to be examined in comparison with other regional *P. infestans* populations.

#### **Evaluation of the microbial quality of cantaloupe fruit produced on raised or flat beds following a flooding event**

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Floodwaters can harbor human and plant pathogens and present a major risk of contamination to fresh fruit and vegetable production in Louisiana. This study evaluated the effect of flooding on the microbial quality and safety of cantaloupe in an agricultural setting. Fields (raised or flat bed) containing mature cantaloupe fruit were flooded with surface water spiked with generic *Escherichia coli* ATCC strains 23716, 25922, and 11775. Generic *E. coli* levels on the surface of cantaloupe were enumerated at 24, 48 and 72 hr after flooding. Incidence of *Sclerotium rolfsii* and *Phytophthora capsici* was measured weekly beginning 7 days prior to flooding. A significant reduction of generic *E. coli* was observed on the surface of cantaloupe 24, 48 and 72

hr post flooding on fruit harvested from flat beds but only after 72 hr on the fruit from raised beds. Coliform levels remained the same over the 72 hr on fruit from both bed types. After 72 hr *E. coli* populations were reduced by an average of 1.8 and 2.5log MPN on fruit harvested from raised beds and flat beds respectively. No differences in *S. rolfesii* or *P. capsici* incidence on fruit from flat compared to raised beds were observed. However, disease incidence was significantly higher in flooded plots compared to non-flooded plots. These results provide science-based data that can be used to develop good agricultural practices programs to minimize food safety and plant disease hazards in flood stricken production regions.

#### **Weather patterns associated with Gibberella Ear Rot of Corn in Ohio**

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Gibberella ear rot (GER), caused by *Fusarium graminearum* is an important disease of corn. Since outbreaks of GER are sporadic, understanding the weather patterns associated with this disease is fundamental for GER risk assessment. The objective of this study was to determine the association between weather conditions and GER intensity. During 2015, 15 hybrids were tested at 10 locations in Ohio. At the R1 growth stage, ten arbitrarily-selected primary ears per hybrid were inoculated in the silk channel with 2 ml of a spore suspension containing  $2.5 \times 10^5$  macroconidia of *F. graminearum* per mL. Air temperature, relative humidity (RH), surface wetness, and rainfall data were collected at 15-min intervals using local weather stations. At R6 all inoculated ears plus five non-inoculated ears were hand-harvested to estimate the incidence and severity of GER. Symptomatic infection was not observed on non-inoculated ears of any hybrid at any location, indicating that natural infection likely did not occur. Two locations had relatively high GER incidence (18 to 25%), four had low incidence (0.7 to 1.4%), while incidence was zero at the other locations. A preliminary descriptive analysis of the data indicated that GER was associated with 3 to 5 rainy days during the 7 days before R1, moderate temperatures (approximately 20°C) during R1, and RH above 80% during the week after R1.

#### **Evaluating soybean production in fields infested with *Heterodera glycines* and *Macrophomina phaseolina* with spatial regression analyses**

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*Heterodera glycines*, the soybean cyst nematode, and *Macrophomina phaseolina*, causal agent of charcoal rot, are economically important soybean pathogens. The spatial distribution of both pathogens was previously described as aggregated. The objective of this study was to use and compare ordinary least squares (OLS) and spatial regression models to evaluate soybean production in fields co-infested with these pathogens and to assess their role in soybean performance. Six trials were conducted in fields naturally infested with *H. glycines* and *M. phaseolina*. Within 30 days after planting, a  $10 \times 10$  grid of contiguous quadrats ( $7.62 \times 7.62$  m) was marked in each field. Fifteen to 20 soil cores were collected at each grid for initial (Pi) and final (Pf) *H. glycines* egg and *M. phaseolina* colony-forming unit (CFU) counts, respectively. Twenty-five plants were collected and hand-threshed from every grid for yield analysis. Moran's *I* test indicated that yields from five fields were spatially correlated ( $P < 0.05$ ). In these fields, spatial error and spatial lag regression models were fitted and compared with the OLS model, which does not account for spatial correlation. Spatial regression analyses revealed a significant interaction effect between *H. glycines* and *M. phaseolina* on soybean yield for fields with high initial Pi of both pathogens. Results from this study will aid in long-term disease management and evaluation of pathogen interaction in reducing crop production.

#### **A model for predicting onset of Stagonospora nodorum blotch in winter wheat based on pre-planting and weather factors**

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Stagonospora nodorum blotch (SNB), caused by *Parastagonospora nodorum*, is a serious disease of wheat. Its management relies mainly on fungicide application after flag leaf emergence. The disease can occur prior to flag leaf emergence but the impact of onset time on yield has not been determined. Using 390 disease cases collected across 11 counties in North Carolina from 2012 to 2014, the effect of SNB onset on yield was analyzed to identify a disease onset threshold. A model was then developed to predict the time of SNB onset using pre-planting factors and cumulative daily infection values (*cDIV*) starting 1 to 3 weeks prior to the disease onset threshold. Time of disease onset explained 32% of the variation in yield ( $P < 0.0001$ ), and day of year (DOY) 102 was identified as the disease onset threshold. Below-average yield occurred in 87% of the disease cases when onset occurred before DOY 102, but only in 28% of those cases where onset occurred on or after DOY 102. A binary logistic regression model indicated that *cDIV* accumulated until two weeks prior to DOY 102 and wheat residue were significant ( $P < 0.0001$ ) predictors of SNB onset. The model had a correct classification rate of 0.94, and specificity and sensitivity rates were  $\geq 0.91$ . Internal model validation based on two cross-validation techniques was also high. The model could serve as a useful decision support tool for fungicide application for SNB management in wheat.

#### **Incidence and severity of Phytophthora disease and assessment of inoculum levels in Texas citrus orchards**

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*Phytophthora* foot and root rot disease, caused primarily by *P. nicotianae*, is present in Texas citrus orchards but there is a lack of information on the incidence and severity of the disease in the State. In addition, Texas citrus growers lack information on relative levels of *P. nicotianae* inoculum which are needed to make treatment decisions based on established thresholds. To address these knowledge gaps, a survey was conducted from February to June of 2015 across the three-county of Rio Grande Valley region where commercial citrus orchards in Texas are located. Twenty-four grapefruit, two tangerines and four sweet orange orchards were surveyed. 20 trees per orchard were rated for foot rot and gummosis lesion severity on a scale of 0 (no lesion) to 5 (entire trunk covered with lesion and gummosis). The survey revealed that 96% of Texas citrus groves are affected by foot rot and gummosis with the disease severity index ranging from 1 to 57%. Further analysis revealed that 20% of the surveyed citrus orchards had more than 50% disease incidence with an average disease severity of 23.6%. *Phytophthora* propagule counts in the soil ranged from less than, five to  $118 \text{ cfu/cm}^3$  with 46% of orchards having more than the reported damaging levels of 20  $\text{cfu/cm}^3$ . The data will be useful to growers for making treatment decisions and in predicting yield losses in the region due to foot rot and gummosis.

#### **Potential global distribution of blueberry twig blight (*Diaporthe vaccinii*) predicted by two species distribution modeling approaches**

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Blueberry twig blight (*Diaporthe vaccinii*) is an endemic disease in parts of the USA and Canada. It has been detected in limited locations in Belarus, Chile, China, Europe and Russia, but was reported to be eradicated in Chile and most of Europe, where it is a quarantine organism. Models on the potential spread of *D. vaccinii* do not exist. Published data on its worldwide occurrence were inventoried and supplemented with data from the National Plant Diagnostic Network. Occurrence and long-term climate data were entered in the species distribution models MaxEnt and Multi-Model Framework. The models correctly predicted that the climate in central-eastern USA, the US and Canadian west coasts, Latvia, Belarus and western Russia would be conducive to blueberry twig blight. Large areas in Europe, Eastern Australia, New Zealand, and smaller areas in South America and East Asia also were predicted to be conducive. Precipitation in the driest quarter contributed most to the prediction, followed by mean annual temperature, with an optimum of 10°C. The potential impact of *D. vaccinii* in Europe and its potential spread to native *Vaccinium* species is unknown.

The pathogen can be spread by natural and human-assisted means, and the risk of introduction, establishment and spread of *D. vaccinii* along different pathways, as well as potential impacts and the efficacy of risk reduction measures, will be assessed by the Plant Health Panel of the European Food Safety Authority.

#### **Symptomatology and epidemiology of Exobasidium leaf and fruit spot of blueberry**

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Over the past decade, Georgia has become one of the largest producers of cultivated blueberries in the United States. As production has expanded, several new blueberry diseases have emerged, with *Exobasidium* leaf and fruit spot caused by the fungal pathogen *Exobasidium maculosum* being the most recent. The disease causes early-season leaf spotting and green spots on fruit. The pathogen produces both basidiospores and yeast cells, but its life cycle, including its overwintering biology and primary and secondary inocula, is unknown. Epidemiological field studies initiated in 2014 included the use of trap plants, a leaf spot demography study, spore trapping, and epiphytic population enumeration. Monitoring of surface populations showed that *E. maculosum* is capable of overwintering epiphytically on all blueberry tissues tested during dormancy. Demography and trap plant data collected in 2015 suggested that dispersal of primary inoculum occurs shortly after leaf/flower emergence, and that infection of primarily young and tender tissue is favored by prolonged rainy periods (>3 days/week). Disease progress in 2015 indicated that the disease is active from March through late May, and that initial fruit and leaf infection occur simultaneously. Additional observations revealed a novel symptom, lesions on emerging shoots that cause girdling and blighting. Although initial results suggest that the disease is monocyclic, further confirmatory studies are needed.

#### **Effect of soybean canopy closure on Sclerotinia sclerotiorum apothecia production, ascospore release, and primary plant infection of soybean**

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Identifying the best timing for fungicide applications to get the optimum white mold control poses a major challenge to soybean producers. This study investigated the impact of canopy closure on apothecia development and ascospore release, to optimize fungicide application timing. In 2015, 0.355 m and 0.762 m row spacing replicated soybean plots were established at the MSU Montcalm Research Farm. The foliar fungicide Endura (boscalid) was applied to treated plots at the beginning of flowering, R1. The number of apothecia, ascospores, and the disease severity were monitored two times/week. Apothecia were first observed in the 0.355 m plots at full flower, R2, 8 d before they were observed in the 0.762 m plots, at beginning of pod development, R3. The number of apothecia was 50 times higher in the 0.355 m plots than in the 0.762 m plots. Incubated plants were symptomatic up to 13 and 18 days prior to symptom development in the 0.355 m and 0.762 m plots, respectively. There were no significant differences between non-treated and Endura treated plots in terms of final disease intensity and yield. However, there was a significant delay in disease severity ( $P < 0.000$ ) between treated and non-treated fungicide plots for the first 5 and 10 d after fungicide application in the 0.355 m and 0.762 m plots, respectively. There was a quantitative relationship between the number of apothecia and the soil temperature and between the number of apothecia and canopy closure.

#### **Modeling regulatory decisions in response to exotic pest detections: Pest case studies for decision makers**

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Detections of exotic pest require responses from decision makers. Response options include management actions such as: eradication, containment, localized control, or the decision to not implement a program. When uncertainty is high, situational context can drive decision-making, leading to responses that are counter intuitive to epidemiological principles of infectivity, symptom expression, and pathogen spread. Spatially explicit models like Webidemics or spatially implicit models like ExPAT are used to model outcomes of decisions, reducing the reliance on highly uncertain information. These tools are applied based on the situation presented by the particular case study. Based on multiple case studies of all pathogen groups, we will present a matrix highlighting for decision-makers which tools are best suited for different exotic pest detections. In the future, the selection and parameterization of proper tools will supply decision makers with quantitative data to evaluate exotic pest detections.

#### **An impact network analysis for management of Xanthomonas wilt of banana: Adoption thresholds for regional success of single diseased stem removal**

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*Xanthomonas* wilt of banana (BXW) is a major challenge to production for smallholder farmers of Central and Eastern Africa. Lack of resistant varieties and the often high rate of disease spread necessitate timely management. Single diseased stem removal (SDSR; removal at soil level of all visibly infected plants) is a promising approach to management requiring lower labor input compared to the previously advocated whole mat removal. We evaluate SDSR in a regional context using a new framework, Impact Network Analysis (INA). An INA is an evaluation of how technologies can have system-level impact through linked socioeconomic and biophysical networks. Estimates of short-term success rates for on-farm application of SDSR are available for several locations. INA includes projection of long-term regional outcomes based on rates of SDSR adoption. The risk of spread by garden tools is a given, but successful BXW management strategies vary by region. In most highland areas of eastern DR Congo, there is lower insect transmission risk associated with AAA type highland cooking/beer bananas. There, a regional strategy for community-level management does not depend on collective action; individual farmers can effectively manage BXW using SDSR, even if their neighbors do not manage effectively. By contrast, in most lowland areas, collective action is necessary for management, especially in zones planted to ABB type cooking/beer bananas highly susceptible to insect transmission.

#### **Seedborne inoculum thresholds of Pseudomonas syringae pv. aptata, causal agent of bacterial leaf spot, in 'baby leaf' Swiss chard crops**

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Most US table beet and Swiss chard seed production (~90%) occurs in western Washington and Oregon. *Pseudomonas syringae* pv. *aptata* (Psa), the cause of bacterial leaf spot, has been identified in beet and chard seed from this region, but seedborne inoculum thresholds have not been established. Chard seed naturally infested with Psa at  $8.6 \times 10^3$  CFU/g was mixed with seed of the same cultivar infested at  $<10$  CFU/g to generate a log<sub>10</sub> series of infestation. Five replicate plots/infestation level were planted in a field in Mount Vernon, WA at  $\sim 10.7 \times 10^6$  seeds/ha in May 2015 in a randomized complete block design. Bacterial leaf spot incidence was  $52 \pm 18\%$  in plots planted with seed infested at  $8.6 \times 10^3$  CFU/g vs. 15 to 21% in plots sown with seed infested at  $<10^3$  CFU/g. Seed infestation level did not affect plant stand or amount of Psa recovered from leaves onto KBC medium. The trial was repeated in the fall, when moist, cool conditions resulted in a leaf spot incidence of  $93 \pm 2\%$  across all plots, and Psa was recovered from every plot ( $9.97 \times 10^{11} \pm 1.82 \times 10^{11}$  CFU/g dry leaf tissue), with no differences among seed infestation levels. Plots sown with seed infested at  $8.6 \times 10^3$  CFU/g had 33% fewer plants, 29% less leaf dry weight, and 126% more severe leaf spot than plots planted with seed infested at  $<10^3$  CFU/g. Psa was transmitted from infested chard seed, even at  $<10$  CFU/g. Environmental conditions in the two trials affected seed transmission and leaf spot severity.

### **Multilayer Networks Supporting Healthy Seed Systems for Smallholder Potato Producers in Tungurahua, Ecuador**

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The potato seed system is a multilayer network that includes social, economic, and agroecological components. Producers, breeding institutions, markets, technical support, and diseases are components of this complex system that interact and modify each other. We studied the structure of the networks of seed sources, markets, and IPM information sources, to understand impacts on the potato seed system of the Consortium of Small Potato Producers (CONPAPA) in Tungurahua, Ecuador. Using information about the structure of the seed system, we can consider how resilient the system is likely to be to stressors such as the introduction of a new pathogen. Which types of farmers may be more vulnerable to losses to such new stressors? One development goal is for there to be good access to quality seed and quality management recommendations, regardless of gender or farm size. Female farmers reported fewer diseases causing seed degeneration than male farmers, and fewer sources of IPM recommendations among the sources less likely to have commercial biases. Identification of key nodes that influence the success of seed systems (e.g., farmers, information, and seed sources) supports enhancement of the system, for improvements such as maximizing the distribution of good varieties, managing outbreaks, and targeted improvement of communication and infrastructure.

### **Genetic diversity of *Pseudomonas syringae* causing bacterial leaf spot on table beet (*Beta vulgaris*) and Swiss chard (*Beta vulgaris* subsp. *cicla*)**

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Table beet and Swiss chard are grown throughout the US, with a significant proportion of baby leaf production in California and 90% of US seed production in Oregon and Washington. Bacterial leaf spot on beet and Swiss chard, caused by *Pseudomonas syringae* pv. *aptata* (Psa), has been reported from California and Oregon, but not yet from Washington. Since 2001, pseudomonads were isolated from surface-disinfested, symptomatic beet and chard plants grown in the western US, including Washington. Strains were variable for fluorescence on KMB agar medium, indicating populations may be diverse. Genetic diversity of the 99 strains was evaluated by repetitive extragenic palindromic PCR (rep-PCR) assay using the BOX AIR primer. Strains from a single Washington outbreak from a chard crop planted with infested seed had DNA fragment-banding patterns identical to the pathotype strain of Psa. The majority of the other isolates were identical, with a pattern similar to but distinct from the pattern of the pathotype. Three additional DNA fragment-banding patterns were observed. Strains from single outbreaks were represented in each of the alternative banding patterns. Preliminary multilocus sequence analysis and pathogenicity tests suggest a majority of bacterial leaf spot outbreaks on chard and table beet in California, Oregon, and Washington are caused by haplotypes distinct from Psa.

### **Diversity and phylogeny of moko disease-causing *Ralstonia solanacearum* strains in Colombia**

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*Ralstonia solanacearum* is a soilborne plant pathogen that causes bacterial wilt on a wide range of hosts of economic importance. This disease is particularly devastating in *Musa* spp., where it is known as Moko disease. A major challenge in controlling this disease is to understand the diversity of the pathogen and how it adapts over time. With such knowledge, it may be possible to identify drivers of adaptation and inform changes in management practices. We therefore sought to characterize the genotypic and phenotypic diversity of *R. solanacearum* isolates obtained from plantations of *Musa* spp. in Colombia, and infer their phylogenetic relationship. Sixty-six isolates were collected. The genetic diversity and phylogenetic relationship among all the isolates were assessed by multiplex PCR and sequence analyses (*mutS*, *rplB*, and *egl*). Multiplex PCR assigned all the strains to phylotype II/sequence 4 and 6. Similarly, the phylogenetic analysis of the gene sequences confirmed the classification of the strains into phylotype II; but, in addition, identified three separate clusters: IIA/6 (banana), IIB/4 (banana), and IIB/4 (plantain). Pathogenicity tests showed symptoms of wilt on banana plants after one month, but only strains isolated from plantain infected tomato. This study shows that plantain and banana plantations in Colombia harbor genetically diverse strains of *R. solanacearum*, belonging to at least three separate clades, and with differences in pathogenicity.

### **Phylogenomic analysis supports polyphyly in *Pythium sensu lato***

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*Pythium* is a large and morphologically diverse genus that includes economically costly root pathogens. Molecular phylogenies support the morphological clustering of *Pythium* into three monophyletic groups: Clades A-D are characterized by filamentous sporangia, clades E-J by globose sporangia, and clade K has been reassigned to the new genus *Phytopythium*. However, different markers have supported different relationships among these clades and to the genus *Phytophthora*. The absence of a robust phylogeny has prevented advances in *Pythium* systematics and evolutionary genomics. In the last six years, whole genome sequences have been generated for seven *Pythium* species and ten other oomycetes. We conducted a phylogenomic analysis using the nucleotide sequences of 277 orthologous genes found in the 17 analyzed oomycete genomes to examine the evolutionary relationships among the major clades of *Pythium*. Our results supported the position of *Phytopythium vexans* as a sister clade to *Phytophthora*. As expected, the remaining *Pythium* taxa formed two monophyletic groups. One group was composed of three taxa that correspond to *Pythium* clades A, B and C, and the other group contained taxa representing clades F, G and I, which was consistent with previous *Pythium* phylogenies. However, the group containing *Pythium* clades F, G and I formed a sister group to the *Phytophthora-Phytopythium* clade and thus *Pythium* clades F-J are not monophyletic.

### **Identification of essential protein machinery involved in the plant disease resistance-signaling node in *Arabidopsis*, NDR1**

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Plant disease epidemics result in an estimated \$200 billion in crop losses globally each year. To understand how plants and pathogens interact, and moreover, to develop a fundamental understanding of the mechanisms that drive pathogen virulence and adaptation, research in our group is focused on the molecular-genetic processes underpinning host resistance. To do this, we use the *Arabidopsis thaliana* – *Pseudomonas syringae* pv. *tomato* (*Pst* DC3000) pathosystem to study the role of non-host disease resistance-1 (NDR1), a key regulator of the of the disease signaling network of CC-NB-LRR (coiled-coil nucleotide binding leucine rich repeat) resistance (R) proteins. In the current study, we present a comprehensive analysis, utilizing a combination of genetic-, biochemical- and cell biology-based approaches to identify and define the NDR1 interactome. In brief, we utilized immunoprecipitation methods, coupled with liquid chromatography mass spectrometry, to identify a list of candidate interacting proteins. Through this approach, we have identified a family of proteins that interact with NDR1 directly *in planta*, and have begun to characterize the role of these interactions in the NDR1-dependent resistance-signaling pathway. Data will be presented that provides support for a model that posits NDR1's association with key, Rho-related GTPases from plants (ROPs), plasma membrane-localized signaling proteins involved in the resistance to *Pst* DC3000.



### Examining putative motility genes in *Clavibacter michiganensis* subsp. *michiganensis*

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The Gram-positive bacterium *Clavibacter michiganensis* subsp. *michiganensis* (*Cmm*) is the causative agent of bacterial canker of tomato (*Solanum lycopersicum*). *Cmm* historically has been characterized as a non-motile pathogen, however, it is known to move acropetally and basipetally in the xylem of infected plants. Under artificial xylem conditions in microfluidic chambers, non-flagellated *Cmm* actively moved against the media flow at ~6  $\mu\text{m}/\text{minute}$ ; these speeds were similar to those observed in *planta*. We have hypothesized that *Cmm* uses a type IV pilus (Tfp) mediated motility system similar to that of *Xylella fastidiosa*. Tfp are diverse in that biosynthesis can be mediated by *pil*, *com*, and/or *tad* operons in both Gram-negative and positive bacteria. Two tight adhesion (*tad*) operons have been identified through sequence similarity in the annotated genome of *Cmm*, and literature suggests they may play a crucial role in biosynthesis of Tfp. *Cmm* transformants were generated by disrupting the *tad* operon promoter. Microfluidic chambers will be utilized to mimic *in planta* conditions of xylem flow to assay and compare motility between mutants and wildtype. Genetic and microscopic studies will be complemented with *in planta* virulence assays to investigate the role of *tad* genes in the *Cmm*-tomato pathosystem. Understanding how the pathogen infects and moves in the plant will strengthen our biological knowledge of *Cmm*, possibly leading to novel disease control strategies.

### Differential effects of *Fusarium thapsinum* on host nitric oxide (NO) synthesis among stalk rot resistant and susceptible sorghum lines

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*Fusarium thapsinum* (FT) is a hemibiotrophic fungus causing stalk rot in sorghum (*Sorghum bicolor* (L.) Moench). NO is a powerful oxidant involved in programmed cell death and FT-mediated NO synthesis was investigated in host stalk tissues. Two greenhouse-grown stalk rot resistant (SC599, SC35) and susceptible (Tx7000, BTx3042) sorghum lines were inoculated with FT and phosphate-buffered saline (control). At 7 and 30 d post-inoculation (d.p.i.), FT- and mock-inoculated stem cross sections were treated with 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate, and observed under a confocal microscope (excitation 488; emission 515 nm). The fluorescence generated by controls was used as the baseline. At 7 d.p.i., strong green fluorescence was observed in FT-inoculated resistant lines, confirming the presence of NO, while no (or weak) signal was evident at 30 d.p.i. The susceptible lines revealed the opposite phenomenon, except for the signal detected in BTx3042 at 7 d.p.i. These patterns suggested that FT switches from biotrophy to necrotrophy by 30 d.p.i. While the presence of NO in SC599 and SC35 at 7 d.p.i. contributes to resistance against the biotrophic phase, its absence at 30 d.p.i. may decrease vulnerability to the necrotrophic phase. Tx7000 showed the opposite response and is susceptible to both phases of the pathogen's lifestyle. Based on NO response, BTx3042 appeared to be moderately resistant to the biotrophic phase but prone to the necrotrophic phase of FT.

### Stalk sugar content and charcoal rot disease reaction in grain sorghum

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Charcoal rot, caused by *Macrophomina phaseolina* (MP), is an important fungal disease in grain sorghum (*Sorghum bicolor* (L.) Moench). An RNA-Seq experiment was conducted to explore the molecular basis of the sorghum-MP interaction. Stalk RNA extracted from MP- and mock-inoculated resistant (SC599) and susceptible (Tx7000) sorghum lines at 2, 7, and 30 d post-inoculation (d.p.i.) was subjected to RNA-Seq. Genes involved in UDP-glucose conversion, sucrose degradation to ethanol and lactate, and fructose degradation to pyruvate and lactate metabolism were significantly up-regulated in Tx7000 at 7 d.p.i., while those of SC599 were not differentially expressed. To confirm the results, stalk juice was extracted at 7 d.p.i. from two MP- and mock-inoculated resistant (SC599, SC35) and susceptible (Tx7000, BTx3042) lines and subjected to HPLC. Concentrations (mg/mL) of sucrose, glucose, and fructose in stalk juice of SC599 (95.3, 23.9, 20.7, respectively) and SC35 (66.2, 31.2, 23.4) were significantly greater than those of Tx7000 (2.7, 6.5, 4.1) and BTx3042 (9.1, 10.9, 6.9) across MP- and mock-inoculations ( $P < 0.0001$ ). Moreover, the reduction of sucrose, glucose, and fructose in MP-inoculated susceptible lines was 86.4, 26.5, and 19.8%, respectively, while those of the resistant lines were 17.0, 21.5, and 7.3%. Therefore, sorghum genotypes with higher stalk sugar content along with lesser sugar reduction under MP infection appeared to be more resistant to charcoal rot disease.

### Spectral signatures of chemical changes in the wheat cell-wall resulting from stripe rust infection in compatible and incompatible near isogenic lines

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Wheat stripe rust, caused by *Puccinia striiformis* f. sp. *tritici*, is one of the most devastating diseases in North America. The biotrophic fungus produces uredia, consisting asexual urediospores, on wheat leaves along the veins and obtains nutrition from host through haustoria. In a compatible interaction, the fungus damages the cell-wall in a susceptible cultivar, however in a resistant cultivar the incompatible interaction occurs as a result of hypersensitive cell death in the host. The purpose of this study was to understand the biomolecular changes in cell-wall compounds in each of the interactions. Two Avocet lines: one carrying *Yr10* and other susceptible (no resistance allele) were inoculated with two isolates (one virulent on both and the other virulent only on *Yr10*). Infected and freeze dried leaves collected at 1 day and 15 days post inoculation were assessed by Fourier transform infrared (IR) spectroscopy. A significant difference in disease severity was observed between the two wheat lines. Principal component analysis of the IR spectra indicated substantial differences in the cell-wall components of the two wheat lines before and after pathogenic infection. There was a significant difference in the biomolecular changes between compatible and incompatible interactions. The most important IR peaks in the leaf cell wall that contributed to such differences were assigned to phenolic and aromatic rings, and proteins.

### Small RNA processing in *Sclerotinia sclerotiorum*

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RNA silencing pathways are under-studied in plant pathogenic fungi. Once elucidated, they can be utilized to improve plant health by more effectively silencing fungal genes from host plants or viral vectors. In this study, we characterized changes in gene expression and small RNA accumulation in *Sclerotinia sclerotiorum*, a damaging and widely distributed fungal plant pathogen, in response to hypovirus infection. Our results indicated that the fungus produced small RNAs of predominantly 22 nt. The *S. sclerotiorum* genome was predicted to contain 59 families of microRNA-like (miRNA) genes, which similar to other recently characterized fungal species, were poorly conserved with other eukaryotes. In degradome analyses, 39 of the miRNAs sequences were predicted to mediate cleavage of 47 *S. sclerotiorum* mRNAs. Consistent with the role of RNA silencing in defense against mobile and infectious genetic elements, most of the small RNA sequences in virus-free *S. sclerotiorum* were derived from retrotransposons. Similarly, large amounts of predominantly 22 nt sequences were detected from the hypovirus genome, indicating that it also was targeted for degradation by host RNA-mediated antiviral defenses. These findings suggest that virus-induced hypovirulence could result from disruption of RNA silencing and that a robust RNA silencing pathway exists in *S. sclerotiorum* that can be exploited for RNA-based pathogen resistance strategies.

**Investigation on the role of molecular composition and lignification of canola plant cell wall in resistance mechanisms associated with CR gene *Rcr1***

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Clubroot disease, caused by *Plasmodiophora brassicae* Woronin, is a serious soil-borne disease of crucifer crops worldwide. It poses a serious threat to canola production in western Canada. *Rcr1* is a new clubroot resistance gene identified in *Brassica rapa*. A multifaceted approach was employed to understand the molecular changes in canola plant cell wall associated with *Rcr1*-mediated clubroot resistance and the expression of defense-related genes involved in the lignin pathway. Fourier Transform mid Infrared (FTIR) spectral analysis showed that non-inoculated canola roots from both resistant (R) and susceptible (S) populations were grouped together and distinctly separated from those inoculated with *P. brassicae*. R and S plants responded differentially to the infection; it appeared that the cell-wall components of roots were changed for plants carrying *Rcr1*, including lipids, proteins, lignin, aromatic and phenolics ring, polysaccharides, and carbohydrates. qPCR identified the upregulation of nine defense-related genes involved in the lignin pathway, as well as in chitinase and xyloglucan endo-transglycosylase activities in roots carrying *Rcr1* relative to those of non-inoculated, but the level of increase was more pronounced with plants carrying *Rcr1*. The changes cell-wall components may reflect differential regulation of genes involved in host defense responses, in particular, the lignin pathways and chitinase synthesis mediated by *Rcr1* during clubroot resistance.

**A sensitive method for quantifying cercosporin, a fungal-derived secondary metabolite, in plant tissue**

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*Cercospora* species infect a wide range of host plants, including soybean and maize. Many species of *Cercospora* produce the phytotoxic secondary metabolite cercosporin. The role of cercosporin as a virulence factor has been demonstrated in several *Cercospora* species. However, it is not clear whether cercosporin is important for virulence in all species of *Cercospora*. To gain a more complete understanding of the role of cercosporin in plant pathogenesis, sensitive methods for its detection and quantification are required. Therefore, the objective of this research was to develop a mass spectrometry based method for quantification of cercosporin from plant tissues with high sensitivity. To this end, extraction, sample cleanup, chromatographic separation, and mass spectrometry based detection methods were developed and optimized. The use of liquid chromatography coupled with a triple quadrupole mass spectrometer allowed detection of cercosporin at concentrations as low as 1 ng/ml. Currently, the feasibility of using this method for quantifying cercosporin in soybean leaves and seed infected with *C. kikuchii* and maize leaves infected with *C. zea-maydis* is being examined. A sensitive method for detecting and quantifying cercosporin will provide a valuable tool for determining the role of this compound in virulence among *Cercospora* species.

**Role of *Cryphonectria parasitica* Cdc48-like gene, CpCdc48 in regulation of growth & cell division**

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Functional analysis of a cell division cycle 48 (CDC48) ortholog, CpCdc48, from *Cryphonectria parasitica* was performed via construction of a CpCdc48-null mutant. Genotype analysis revealed that the putative CpCdc48-null mutant was a heterokaryotic transformant containing two different types of nuclei (i.e., one with the wild-type CpCdc48 allele and the other with the CpCdc48-null mutant allele). Although stable mycelial growth of the heterokaryotic transformant was observed on media containing hygromycin B, neither germination nor growth of the resulting spores was observed on selection media, suggesting that the CpCdc48 gene is essential. Microscopic analysis of germinated conidia from the heterokaryon demonstrated that although there were normal germinating spores due to the wild-type conidia, there were many residual conidia that did not germinate. However, with prolonged incubation, non-germinating conidia began to swell into gigantic globose spores. DAPI staining and FACS analysis of the gigantic spores revealed the presence of multiple nuclei. These gigantic conidia did not show any signs of polarized growth and underwent autolysis with further incubation. These findings indicate that the CpCdc48 gene is responsible for delayed cell cycle during spore germination, resulting in some karyokinesis, but not following spore cytokinesis. Thus, CpCdc48 is essential for cell division and polarized growth.

**Wheat streak mosaic virus-encoded N1a-Pro and coat protein are involved in virus superinfection exclusion**

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Cross protection or superinfection exclusion (SE) is defined as the phenomenon whereby initial infection by one virus prevents subsequent infection by closely related viruses. The mechanisms of SE are just beginning to be understood. *Wheat streak mosaic virus* (WSMV; genus: *Tritimovirus*; family: *Poymoviridae*) is an economically important wheat virus. Proteins encoded by WSMV were individually expressed in wheat using an unrelated *Triticum mosaic virus* (TriMV) gene expression vector, followed by challenge inoculation with GFP-tagged WSMV (WSMV-GFP). Wheat plants infected with TriMV expressing WSMV P1, HC-Pro, P3, 6K1, CI, 6K2, N1a-VpG or N1b cistrons did not prevent superinfection by WSMV-GFP. However, expression of WSMV N1a-Pro or CP greatly reduced superinfection by WSMV-GFP, suggesting that N1a-Pro and CP are involved in SE. Expression of WSMV N1a-Pro or CP cistron with frameshift mutations did not exhibit SE against WSMV-GFP, indicating that the N1a-Pro and CP proteins but not the RNA sequences are involved in SE. A series of deletions comprising 60 amino acid (aa) sets covering the entire N1a-Pro cistron revealed that the complete N1a-Pro protein is required for SE activity. In contrast, WSMV CP aa 3-100 and 301-349 were dispensable for SE but not aa 101-300. Expression of WSMV CP aa 101 to 300 in TriMV exhibited the same SE activity as full-length CP. In reciprocal experiments, wheat infected with WSMV expressing TriMV CP prevented superinfection by TriMV-GFP.

**In planta interaction between *Tobacco mosaic virus* 126 kDa protein and a host membrane protein (SNARE) and its influence on virus RNA accumulation**

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*Tobacco mosaic virus* (TMV) 126 kDa protein aids virus accumulation and is necessary for virus intercellular movement. This protein also co-localizes or associates with the endoplasmic reticulum and vacuole membrane (reviewed in Liu et al. 2013 Front. Plant Sci. 4:12. doi: 10.3389/fpls.2013.00012). The importance of the 126 kDa protein interaction with different host membranes as it relates to virus accumulation is not understood. As shown through yeast two hybrid analysis the 126 kDa protein interacts with two host proteins: one putatively involved in membrane fusion with vacuoles and the second with intra-vacuolar protease activity. Here we will show the co-localization of the membrane fusion protein, a plant SNARE (soluble N-ethylmaleimide sensitive attachment protein receptor) protein, *in planta* with the 126 kDa protein. Additionally, we are analyzing the influence of this and other SNARE proteins on tobamovirus [TMV and *Turnip vein clearing virus* (TVCV)] RNA accumulation using Arabidopsis T-DNA mutants. TVCV is being studied because of the different characteristics of its 126 kDa protein homolog, the 125 kDa protein (e.g. different aggregation states displayed during their ectopic expression). The importance of this SNARE protein for subcellular localization of the TMV 126 kDa protein and virus RNA accumulation will be summarized.

### **Overexpression of a modified plant thionin enhances disease resistance to citrus canker and Huanglongbing (HLB, citrus greening)**

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Huanglongbing (HLB or citrus greening disease) caused by *Candidatus Liberibacter asiaticus* (Las) is a great threat to the US citrus industry. Citrus canker is also an economically important disease associated with a bacterial pathogen (*Xanthomonas citri*). In this study, we characterized endogenous citrus thionins and investigated their expression in different citrus tissues. We aim to develop citrus resistant to both HLB and citrus canker through expression of modified plant thionin. In order to improve effective for disease resistance, we modified and synthesized the sequence encoding plant thionin and cloned into binary vector pBinPlus/ARS. The construct was introduced into *Agrobacterium* strain EHA105 and used for citrus transformation. Transgenic Carrizo plants expressing the modified plant thionin were successfully generated through *Agrobacterium*-mediated transformation. Transgenic Carrizo plants expressing the modified thionin gene were challenged with *X. citri* 3213 at various concentrations, and showed a significant reduction in canker symptoms and a decrease in bacterial growth compared to nontransgenic plants. Furthermore the transgenic citrus plants were challenged with HLB via graft inoculation. The results showed significant decrease in the Las titer in transgenic Carrizo compared with nontransgenic plants. These data, thus, provide a promising approach to engineering citrus disease resistance against HLB and canker.

### **Transgenic Carrizo expressing 'Candidatus Liberibacter asiaticus' effector P235 displays Huanglongbing-like symptom and alters some gene expression**

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'*Candidatus Liberibacter asiaticus*' (Las) is the agent causing destructive citrus huanglongbing worldwide. A putative protein (P235) was identified in Las genome prophage region, which encodes a 123 amino-acid protein with a molecular weight of 14.19 kD. Green fluorescence protein (GFP)-fused with P235 appeared to accumulate in *Nicotiana benthamiana* cell nuclei by *Agrobacterium*-mediated transient expression, however, P235-GFP fusion protein in transgenic citrus targets the citrus chloroplast. P235 alone was constructed into a binary vector for citrus transformation. Over 40 independent transgenic Carrizo plants were obtained. Gene integration and expression were confirmed by molecular analysis. We observed that some P235-expressing Carrizo plants displayed HLB-like symptoms with leaf chlorosis and plant growth retardation. P235 expression levels, determined by RT-qPCR, correlated with HLB-like symptoms. Transcriptome analysis revealed that carbohydrate metabolism and biotic stress pathways were significantly affected in transgenic citrus expressing p235 effector. Higher transcript levels were determined for several stress-related genes involved in hormone metabolism and zinc ion binding. This is the first report that '*Candidatus Liberibacter asiaticus*' encodes a protein that targets the chloroplast of plant cells and alters plant-host phenotypes.

### **Bioluminescent imaging as a sensitive tool for evaluation of *Ralstonia solanacearum* infection dynamics in resistant and susceptible pepper lines**

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Bacterial wilt, caused by *Ralstonia solanacearum*, is a major disease affecting pepper (*Capsicum annuum*) production worldwide. In this study, we evaluated the resistance of 100 pepper lines using *R. solanacearum* strain Rs-SY1 (phylogroup I, isolated from sweet pepper in South China) and identified one resistant pepper line, BVRC 1. In order to study the bacterial spatial-temporal distribution *in planta* in real time, we generated a strongly bioluminescent *R. solanacearum* strain (BL-Rs7) using pXX3 carrying *luxCDABE* genes. BL-Rs7 was similar to its parent strain Rs-SY1 in morphology and pathogenicity. Furthermore, luminescence intensity of BL-Rs7 strongly correlated with bacterial population *in planta* ( $R^2 = 0.88$ ). The utility of bioluminescence assay was validated by real-time *in vivo* monitoring of *R. solanacearum* infection dynamics in resistant and susceptible pepper lines BVRC 1 and BVRC 25, respectively, following root inoculation. The luminescence signals were detected in susceptible pepper plants from 3 days post inoculation (dpi), while little signal was detected in roots or stem of the resistant pepper line over 10 dpi and only a very low titer of bacteria was detected in the root. The results suggested that pepper line BVRC 1 exhibits resistance by interfering with translocation and multiplication of *R. solanacearum* in the stem.

### **Transgenic approaches for the genetic improvement of cowpea (*Vigna unguiculata*(L.)Walp) and soybean (*Glycine max*(L.) Merrill)**

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Herbicide-resistant transgenic improvement of Cotyledone explants excised from 3 day old *in vitro* of *Vigna unguiculata* and *Glycine max* were infected with *Agrobacterium tumefaciens* (LBA4404) harboring chitinase (*chi II*) and bar gene. Co-cultivated cotyledon explants were inoculated on MS medium containing B5 vitamins BA, TDZ, L-glutamine, adenine sulphate, basta and cefotaxime. Shoots formed at the proximal end of cotyledons were transferred to MS medium containing B5 vitamins, GA3, basta and cefotaxime for elongation. Elongated shoots rooted in hormone free half strength MS medium. Rooted plants were subsequently established in soil. Transformed plants were confirmed by herbicide resistance and PCR analysis. These ready systems enable us to improve seed qualities and agronomic characteristics by transgenic approaches. In addition, with the accumulation of cowpea and soybean genomic resources, convenient or promising approaches will be requisite for the determination and use of gene function in cowpea and soybean. In this article, we describe recent advances in and problems of cowpea and soybean transformation, and survey the current transgenic approaches for applied and basic research in India.

### **Co-expression of proteins by two virus vectors in the same cells of infected plants**

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Plant viral vectors represent a technology that allows expression of valuable proteins at high levels in a relatively short time. For many purposes it may be desirable to express more than one protein in a single cell but that is often not feasible when using a single virus vector. Such a co-expression strategy requires the simultaneous delivery by two non-competitive viruses to express proteins. Here we report on the use of two agro-launchable virus vector systems based on *Tomato bushy stunt virus* (TBSV) and *Tobacco mosaic virus* (TMV) that both express suppressors to minimize the effects of Argonaute2-mediated silencing. To co-express proteins we used TBSV and TMV each expressing green fluorescent protein (GFP) of different sizes, in addition to co-expressing TBSV-GFP with TMV expressing red fluorescent protein (RFP). The results in *Nicotiana benthamiana* and tomato demonstrated that these two vectors accumulated in the same plant, same leaves, and in the same cells. Therefore, co-expression by these two vectors provides a biotechnological platform for fast and high level expression of potentially valuable proteins that need to form oligomers for activity.

### **Displaying foreign proteins and peptides on the surface of *Pepino mosaic virus* virions**

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*Pepino mosaic virus* (PepMV), a member of *Potexvirus* genus, infects tomatoes and causes only mild symptoms under high intensity light conditions. However, its infection is highly productive and yields a large amount of virions in tomatoes. The flexuous PepMV virions (510 nm x ~13 nm in dimensions) are made of 1290 identical copies of a 25 kDa capsid protein (CP) with exposed N-terminus at the virion surface. Therefore, PepMV virions are ideal nanoparticles to display useful antigens and antibodies fused at the N-terminus of the CP. Using a highly infectious cDNA clone as the backbone, we have developed a virus-based expression system that is capable of displaying foreign proteins and peptides on the surface of PepMV virions. Two unique restriction sites have been engineered immediately downstream the AUG translation initiation codon of PepMV CP to allow convenient and directional cloning of any foreign proteins and peptides into the expression vector. Several 2A self-cleavage peptides from picornaviruses have also been inserted between the restriction sites and PepMV CP to modulate variable ratios of CP fusion proteins and mature CP. m<sup>7</sup>G-capped *in vitro* transcripts synthesized from the vectors are highly infectious, yielding high level expressions of foreign proteins and peptides in inoculated plants. The PepMV-based protein display system is expected to have a wide range of applications in pharmaceuticals and diagnosis.

#### **A single nucleotide polymorphism at the right terminal region of Mexican papita viroid is a virulent determinant factor on tomato**

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*Mexican papita viroid* (MPVd), a pospiviroid, causes serious disease outbreaks on greenhouse tomato in North America. Two dominant genotypes (MPV-S and MPV-M), sharing 93.8% sequence identity, incited striking different symptom expression (severe and mild) on tomato 'Rutgers'. To determine genetic factor that is responsible for differential pathogenicity, using synthetic DNA approach, a series of chimeric infectious clones were generated through progressive replacement of each secondary structural domain in MPVd-M with their corresponding domain of MPVd-S. In plant inoculation assays, a chimera containing the Terminal Right domain of MPVd-S incited severe symptom on tomato similar to that induced by MPVd-S. The other three chimeras incited only mild symptoms similar to that of MPVd-M. A single nucleotide polymorphism in the Terminal Right region between MPVd-M (176U:A<sub>185</sub>) and MPVd-S (174G:C<sub>183</sub>) was identified as the virulent determinant factor for MPVd. This result supports variable pathogenicity functions in different pospiviroids, which may lead us to design a better strategy in managing this emerging viroid disease.

#### **Two complete genome sequences of a new pathovar of *Xanthomonas oryzae* infecting wild grasses provide insight into evolution of pathogenicity**

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*Xanthomonas oryzae* (*Xo*) is a group of plant pathogenic bacteria composed of weak and highly virulent members that are genetically distinct from one another. Strains belonging to different virulent lineages from Africa and Asia and less virulent strains from the US have been used for comparative studies of pathogenicity and establishing effector repertoires. A close relative of *Xo*, *X. campestris* pv. *leersiae* (*Xcl*) was first described in 1957, and causes bacterial streak on the perennial grass, *Leersia hexandra*. *L. hexandra* is closely related to rice, globally ubiquitous around rice paddies, and is a reservoir of pathogenic *Xo*. We used draft and long read, single molecule, real time (SMRT) genome sequences of two strains of *Xcl* from China and Burkina Faso to determine the genetic relatedness of this organism with *Xo*. Novel transcription activator like (TAL) effectors and repeat variable domains (RVD) were discovered. Predicted TAL effector target sequences were identified in the available *L. perrieri* genome. Rice susceptibility gene homologs and unique TAL effector targets were also uncovered. Pathogenicity screening on *L. hexandra* and diverse rice cultivars confirmed that *Xcl* can colonize rice and produces weak not progressive symptoms. Overall, based on average nucleotide identity, effector repertoires and phenotype, we propose to rename *Xcl* to *X. oryzae* pv. *leersiae* and use this parallel system to better understand the evolution of pathogenicity in grasses.

#### **Phytopathogenic interaction between *Herbaspirillum rubrisubalbicans* and different genotypes of Sorghum**

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*Herbaspirillum rubrisubalbicans* is a nitrogen fixing and plant growth promoting bacterium found in association with important grasses, such as maize and wheat. In contrast, this bacterium is a pathogen on some genotypes of *Sorghum bicolor*, being responsible for red stripe disease. The pathogen infects sorghum leaves leading to tissue necrosis, a reduction in photosynthesis and impaired leaf development. Twenty five genotypes of sorghum were inoculated with *H. rubrisubalbicans* with the great majority showing symptoms after 7 days. Bacteria were recovered from portions of the leaf increasingly distal from the site of inoculation. The results showed the spread of both bacteria and symptoms along the expanding leaf. Plants have the ability to mount a defense response to invading pathogens due to recognition of conserved motifs, termed microbe-associated molecular patterns (MAMPs). We tested the ability of MAMP-triggered immunity to protect sorghum plants from *H. rubrisubalbicans* infection. Sorghum leaves were pretreated with chitin and flg22 (a conserved 22 amino acid peptide derived from bacterial flagellin) and then challenged with the pathogen. Evaluation of disease symptoms 5 days after inoculation showed strong plant protection in those leaves pretreated with chitin and flg22. The data suggest that *H. rubrisubalbicans* will be a useful pathogen in which to study the innate immunity response of sorghum, an important grain, forage and bioenergy crop.

#### **Expression of *FLS2s* from 'Nagami' kumquat and 'Sun Chu Sha' mandarin enhance citrus canker resistance in 'Duncan' grapefruit**

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In citrus, flagellin 22 (flg22), a pathogen-associated molecular pattern (PAMP) from *Xanthomonas citri* ssp. *citri* (Xflg22), triggers stronger defense responses in canker resistant genotypes than susceptible ones (Shi et al. Mol. Plant Pathol. 2025, 16:507). We have now identified the citrus genes encoding putative bacterial flagellin/flg22 receptors (*FLS2*). We isolated two genes each from 'Sun Chu Sha' mandarin (*Citrus reticulata*, *CrFLS2-1* and *CrFLS2-2*) and 'Duncan' grapefruit (*C. paradisi*, *CpFLS2-1* and *CpFLS2-2*), a canker resistant and a highly susceptible citrus species, respectively. Only one *FLS2* gene was obtained from the highly canker-resistant 'Nagami' kumquat (*Fortunella margarita*, *FmFLS2-1*). Flanking sequences suggest a rearrangement event resulted in the deletion of *FLS2-2* from the genome of kumquat. Transcriptional analysis indicated that the *FLS2-2s* reached higher expression levels in response to Xflg22 than the *FLS2-1s*, and the induction levels positively correlated with the level of canker resistance. Kumquat showed the highest *FLS2-1* pre-treatment expression level among the citrus species tested. We also transiently expressed *FmFLS2-1*, *CrFLS2-2* and *CpFLS2-2* in leaves of susceptible grapefruit. *FmFLS2-1* and *CrFLS2-2* conferred stronger responses to Xflg22 and reduced canker symptom development when compared to *CpFLS2-2*. We conclude that these two genes are valuable for engineering enhanced canker resistance and possibly other bacterial pathogens.

#### **Homologs of *CsLOB1* can function as disease susceptibility genes in citrus canker**

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The *lateral organ boundary domain* (*LBD*) genes is a plant-specific family of proteins that function as transcription factors for plant growth and development. *Citrus sinensis* *lateral organ boundary 1* (*CsLOB1*) functions as a disease susceptibility (*S*) gene in citrus bacterial canker (CBC). The *Citrus sinensis* genome contains thirty-four *LBD* members. We assessed the potential for additional *LBD* genes in citrus to function as surrogates for

*CsLOB1* and compared host gene expression upon induction of different genes. Using custom designed TAL effectors (dTALes), *CsLOB2* and *CsLOB3* functioned similarly to *CsLOB1* in CBC. Quantitative RT-PCR of mRNA during infection revealed a set of cell wall metabolic genes that are associated with *CsLOB1*, *CsLOB2* and *CsLOB3* expression. The genes may represent downstream genes involved in CBC.

#### **Ca. Liberibacter asiaticus carries a highly conserved, chromosomal CI repressor that binds an early gene promoter in its prophage**

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*Candidatus Liberibacter asiaticus* (Las) is one of three species of bacteria associated with Huanglongbing, arguably the most devastating disease of citrus. The majority of Las strains harbor bacteriophage that are maintained as stable lysogens in psyllids, but begin to enter a lytic cycle *in planta*. Las carries a CI phage repressor (LCI) with internal direct repeats that readily recombine and can create truncated translation products. LCI has been used as an epidemiological marker to follow the movement of Las populations; however, LCI has not been functionally characterized. Las strain UF506 has been maintained in isolation in a containment facility and transmitted from citrus to citrus and to periwinkle via dodder. All five LCI sequences isolated from cured Las strain UF506 in infected citrus encoded a full-length protein. By contrast, all seven UF506 LCI sequences derived from artificially infected periwinkle encoded a truncated version. Mobility shift assays demonstrated that LCI binds its own promoter and an early gene promoter on SC1, indicating potential involvement in lytic cycle activation. Promoter reporters are being evaluated to quantify repression in the presence of the full-length and truncated proteins, and additional potential binding sites are being investigated. If the LCI does function as a phage repressor, it would become a promising target for HLB control, since interfering with this function could trigger the lytic cycle in Las.

#### **The Type II-dependent secretion of virulence factors is necessary for systemic colonization by the xylem-limited pathogen, *Xylella fastidiosa***

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*Xylella fastidiosa* (*Xf*) is a xylem-limited, gram-negative bacterium that causes Pierce's disease (PD) in grapevines. PD symptom progression is highly correlated with systemic colonization of the xylem, which is achieved by bacterial movement via the degradation of pit membranes separating adjacent xylem vessels. Pit membranes are readily dismantled by *Xf* cell wall-degrading enzymes (CWDEs), three of which have been implicated as virulence factors: a polygalacturonase (PG), and two endoglucanases (EngXCA1 and EngXCA2). As pathogenicity was nullified in a PG-deficient mutant, the PG has been a focal point for *Xf* inhibition studies. However, PG expression has proved to be difficult, and we have sought alternative CWDE inhibition targets. A knockout mutation of the Type II secretion system (T2SS) ATPase gene ( $\Delta xpsE$ ) resulted in a complete loss of pathogenicity similar to that displayed by the PG-deficient mutant. As *Xf* lacks a Type III secretion system, it is predicted that virulence factors such as CWDEs are secreted via the T2SS. Secretome analyses of both the *Xf* wild-type and  $\Delta xpsE$  mutant strains had distinct differences in protein composition, and an antibody targeting EngXCA2 revealed that secretion of this endoglucanase is Type II-dependent. These results provide clear evidence that *Xf* relies on the T2SS for secretion of the CWDEs necessary for systemic colonization, and inhibition of this secretion system will significantly reduce *Xf* pathogenicity in grapevines.

#### **The chemotaxis regulator *pilG* of *Xylella fastidiosa* is required for virulence in *Vitis vinifera* grapevines**

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*Xylella fastidiosa* is a Gram-negative, xylem-limited pathogenic bacterium that causes Pierce's disease of grapevines. Type IV pili of *X. fastidiosa* are regulated by *pilG*, a chemotaxis regulator in Pil-Chp operon involving signal transduction pathways. To elucidate the role of *pilG* in twitching motility and pathogenicity, phenotypes of wild type, *pilG*-mutant and a complementary strain were characterized. While *in vitro* studies showed that all tested strains had similar growth curves, mutant *Xf* $\Delta$ *pilG* exhibited a twitching defective phenotype and significant reduction in biofilm formation and cell-cell aggregation. Greenhouse *in planta* experiment further confirmed that grapevines infected with *Xf* $\Delta$ *pilG* showed very mild PD symptoms whereas grapevines inoculated with *X. fastidiosa* wild type and *Xf* $\Delta$ *pilG*-C developed typical PD symptoms. These results demonstrate that *pilG* of *X. fastidiosa* is required for twitching motility and full virulence.

#### **Biological functional Analysis of *tatB* in *Acidovorax citrulli***

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*Acidovorax citrulli* causes bacterial fruit blotch of cucurbits world wide. In order to explore the relationship between Twin-arginine Translocation system (Tat) and pathogenicity of *A. citrulli*, a *tatB* disruption mutant ( $?tatB$ ) and its complement strains were constructed by homologous recombination. The virulence, motility, growth ability, extracellular polysaccharide and cellulase producing ability and biofilm formation of the wild type, mutant and the complement strains were tested to illustrate the influence of Tat system on *A. citrulli*. By comparing the expression of *trbC*, *hrcN*, *Aave\_1810*, *Aave\_0034* and *hrpE* gene with real-time fluorescent quantitative PCR, we explored the relationship between *tatB* genes associated with type III secretion system, Tat system related genes and cell toxicity related genes. The results showed the deficiency of *tatB* weakened the virulence, motility and growth ability of *A. citrulli*, while had no effect on the production of extracellular polysaccharide (EPS), extracellular cellulase and biofilm formation. Real-time fluorescent quantitative analysis showed that the deficiency of *tatB* made the expressions of *trbC* and *hrcN* genes in mutant strain significantly increase, while the expressions of other genes notably decreased. Consequently, the deficiency of functional gene *tatB* in Tat affects some biological characteristics of *A. citrulli*, and ultimately weakens pathogenic ability.

#### **Secretome annotation in the citrus greening bacterial pathogen**

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Citrus greening or also known as Huanglongbing (HLB) is one of most deadly citrus disease in Florida and in the world. The causal agent is the bacterium *Candidatus liberibacter asiaticus* (*CLas*). Plant pathogens secrete molecules that manipulate host physiology to achieve colonization. The main goal of our study was to identify and make available to the citrus community a set of candidate pathogen secreted proteins from *CLas* using genome sequences and *in planta* gene expression. Identified secreted protein candidates can be employed in high throughput functional genomics screens, commonly referred to as effectoromics, to test citrus germplasm for specific pathogen recognition by resistance *R* proteins. Our findings highlight about 10 candidate secreted proteins for pathogen-assisted breeding in citrus for resistance to HLB.

#### **The bifunctional catalase/peroxidase *KatG* is required for pathogenicity of *Xanthomonas albilineans* on sugarcane**

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Survival and multiplication of phytopathogenic bacteria, and disease progression are essentially contingent upon the pathogen's ability to detoxify reactive oxygen species (primarily H<sub>2</sub>O<sub>2</sub>) generated either endogenously through aerobic respiration or by the oxidative burst of plant cells during plant-pathogen interactions. *Xanthomonas albilineans* (Xal) is a gram-negative, xylem-invading pathogen that causes sugarcane leaf scald disease. Two

putative catalase genes XALc\_0265 (*srpA*) and XALc\_2991 (*katE*), and a bifunctional catalase/oxidase XALc\_0959 (*katG*) have been annotated *in silico* in the genome of Xal FL07-1. Xal *katG* was replaced by an enhanced Green Florescent Protein marker gene (*gfp*) via splice overlap PCR. Challenge inoculations of decapitated sugarcane plants by Xal  $\Delta$ *katG::gfp* exhibited a significant reduction in pathogenic symptoms on the newly emerged leaf blades. In addition bacterial viability *in planta* was significantly reduced compared to the wild type Xal FL07-1. Full-length *katG* cloned in the broad host range vector pUFR047 complemented the  $\Delta$ *katG::gfp* mutant strain for both pathogenicity and endophytic survival of the bacterium. Given that Xal *katG* is predicted to have a non-classical secretion potential, its role is also being investigated in dampening the apoplastic, non-specific low amplitude systemic wave of H<sub>2</sub>O<sub>2</sub> elicited by the host cell that is essential for establishment of innate plant immunity.

#### **The role of the tomato genotype in susceptibility to *Salmonella enterica***

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Tomatoes were linked to multiple outbreaks of salmonellosis. A hypothesis that some tomato genotypes are less conducive to *Salmonella* growth was tested. A screen of 30 cultivars revealed 10-1,000 fold differences in the ability of this human pathogen to multiply within fruits. Expression of *Salmonella* genes involved in the interactions was tomato cultivar- and ripening-dependent. *Salmonella* reached lower numbers within immature fruit, however, pigment accumulation *per se* did not have an effect on its growth within tomatoes. *Salmonella* reached similar population densities in tomatoes carrying *green flesh* or *Hyper pigmented* mutations. Growth of *Salmonella* was reduced in the tomato genotypes with defects in ethylene synthesis, perception and signal transduction. While mutation in the ripening-related ethylene receptor *Nr* resulted only in a modest reduction in *Salmonella* numbers within tomatoes, strong reduction of the *Salmonella* growth was observed in *rin* and *nor* tomato mutants. A commercial cultivar Sebring heterozygous for *rin* was less susceptible to *Salmonella* under the greenhouse conditions than other large-fruited varieties. No significant differences in growth of *Salmonella* within green and red tomatoes of cv. Sebring were observed in the greenhouse experiments, consistent with the hypothesis that ethylene signaling is responsible for the phenotype of green tomatoes. However, in the field, the phenotype of the cv. Sebring was masked by environmental conditions.

#### **Determining the genetic basis for stomatal aperture modulation by *Salmonella enterica* serovar Typhimurium strain 14028**

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Pathogens that cause foodborne illness pose a challenge to food safety and security, as crops that are vectors for these human diseases may appear healthy, resulting in their integration to marketplaces throughout the world. Although some human pathogens that are introduced to the phyllosphere elicit an immune response within the plant, *Salmonella enterica* serovar Typhimurium strain SL1344 has been shown to disrupt plant innate immune signaling (including stomatal immunity) and survive for long periods of time inside the leaf. We have determined that, like SL1344, *S. enterica* serovar Typhimurium strain 14028 (14028) also disrupts stomatal immunity after 4 hours post-inoculation (hpi). We set up a genetic screen to elucidate the mechanism(s) by which 14028 modulates stomatal movement using a mutant library with high-coverage and multi-gene deletions created with lambda-red mediated gene-replacement. Leaf sections of lettuce (*Lactuca sativa* cultivar Salinas) were floated on bacterial inoculum (10<sup>8</sup> CFU/mL) or a water control under light and stomatal aperture widths were measured via microscopy at 4 hpi. A total of 535 mutants were analyzed for their inability to stimulate stomatal opening at 4 hpi and compared to the wild type and water control. We observed that mutant strains lacking operons involved in secretion could not open stomata. Further characterization of identified mutants will aim to determine the individual genes that are pertinent for stomatal opening.

#### **In vivo and in vitro investigation of the mobilization of pathogenicity islands of *Streptomyces***

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Approximately ten *Streptomyces* species including the most widely distributed *Streptomyces scabiei* cause the potato scab disease. The main pathogenicity determinant of scab-causing *Streptomyces* species is the phytotoxin thaxtomin A (ThxA). In *S. turgidiscabies*, ThxA biosynthetic genes are localized on a mobile pathogenicity island (PAI). However, the PAI mobilization in other *Streptomyces* species remains uncharacterized. We investigated the mobilization of the PAI of *S. scabiei* 87-22 both *in vivo* and *in vitro*. We sequenced genomes of ten pathogenic *Streptomyces* isolates from different locations, inferred the evolutionary relationships of pathogenic *Streptomyces* species, and identified a novel pathogenic species (*S. sp.* 96-12) that was isolated from Egypt. The PAI in *S. sp.* 96-12 is identical to the PAI in *S. scabiei* 87-22 despite differences in their whole genome sequences. This strongly suggested *in vivo* mobilization of this PAI in these two strains. To test whether the PAI in *S. scabiei* 87-22 could indeed be mobilized, *S. scabiei* 87-22 deletion mutants with antibiotic resistance markers in the PAI, were mated with *S. diastatochromogenes*, a nonpathogenic species. The PAI of *S. scabiei* was found to be site-specifically inserted into the *aviX1* gene of *S. diastatochromogenes* and conferred pathogenicity. Our results demonstrated that the mobilization of the *S. scabiei* PAI could be responsible for the emergence of novel pathogenic species.

#### **Characterization of the T6SS secretion system of *Ralstonia solanacearum* and its role in virulence at low temperatures**

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*Ralstonia solanacearum* is the causal agent of Bacterial Wilt, the worldwide devastating disease of hundreds of plant species. *R. solanacearum* is a 'complex' species due to its genetic diversity and wide host range; however all strains possess conserved secretion systems. The type VI secretion system (T6SS) has been associated with virulence, symbiosis and bacterial competition, however its function is not known in *R. solanacearum*. Previously, we discovered a functional T6SS in several strains of *R. solanacearum*, through a proteomic comparison. We created a T6SS related (Vip-B like) gene defective mutant in strain P673. In this work we confirmed that this mutant renders the T6SS non-functional. We show that the T6SS contributes to P673 virulence at low temperatures, only when soil inoculated. We hypothesized that the T6SS contributes indirectly to virulence, either by competition with other bacterial species, or with populations of the same species. To test this hypothesis we performed bacterial competition assays with other bacterial species commonly found in soil, however we did not find a difference. Currently, we are developing intra-species competition assays at 30°C and 18°C in order to determine the T6SS function and its dependency on temperature. We also present a comparative genomics of the T6SS system for all the strains sequenced to date. Characterization of the T6SS system in *R. solanacearum* will help elucidate its role in virulence at low temperatures.

#### **Global regulatory changes allow for the increased growth of *Salmonella* in soft rotted tomatoes**

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While little is known about the mechanisms that allow *Salmonella* to multiply in tomatoes, even less is understood about how interactions of this human pathogen with phyto-bacteria contribute to its survival in alternate hosts such as plants. It is now known that bacterial soft rot contributes to an increased fitness of *Salmonella enterica* sv. Typhimurium in tomato fruits. The goal of this research is to understand the underlying mechanisms involved in this

phenomenon. The hypothesis is that a change in the tomato environment caused by *Pectobacterium* provides an overall benefit for *Salmonella*. In order to determine the full complement of functions that may be vital for the success of *Salmonella* in soft rots, we employed high throughput transposon sequencing (TN-seq). Libraries of mutants made via transposon mutagenesis were seeded into tomatoes inoculated with fully virulent *Pectobacterium*, less virulent *Pectobacterium*, and no *Pectobacterium*. As a result, pathways involved in nitrogen metabolism, motility, and other regulatory pathways have been identified as potentially playing a role in these interactions. Deletion mutants of genes identified by the TN-seq were then constructed via Wanner-Datsenko mutagenesis for individual testing in tomatoes. These individual mutations have confirmed the results of the TN-seq. We conclude that a variety of metabolic changes do in fact contribute to the increased growth of *Salmonella* in tomatoes.

#### **Expression of the *FST1* from *Fusarium verticillioides* in a yeast strain lacking a major myo-inositol transporter gene**

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*Fusarium verticillioides* causes an important seed disease on maize and produces fumonisin B1 (FB1), a mycotoxin that is detrimental to human and animal health. Previous studies discovered that expression of *FST1* is required for FB1 production. In addition, strains lacking a functional *FST1* are less virulence on maize seeds and more inhibited by hydrogen peroxide than the wild-type strain. The mutation also causes reduced hydrophobicity of hyphae and macroconidia production. *FST1* encodes a putative protein with 12 transmembrane domains with sequence similarity to hexose transporters. However previous studies have failed to prove its ability to transport glucose, fructose or mannose. In this research, we used an *Saccharomyces cerevisiae* strain lacking a functional myo-inositol transporter (*ITR1*). This strain grows poorly in myo-inositol medium and is not inhibited by increasing concentrations of FB1. Expression of *FST1* in the *itr1* mutant restored growth on myo-inositol medium and the inhibitory effects of FB1 to levels observed in the wild-type yeast strain. These results indicate that *FST1* can function as a myo-inositol transporter. The evidence also suggests that *FST1* can transport FB1 in to the fungal cell.

#### **Evaluation of Rice Varieties for Resistance for Autumn Decline in Arkansas**

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Symptoms of autumn decline include black root rotting with stunted and yellowish rice foliage starting as early as two weeks following permanent flood establishment. The problem is often most severe where cold well water first enters a rice field and may spread throughout the field, except on levees. The phenomenon was reported in Arkansas in a limited number of fields in 2004. However, there were several reports of autumn decline across the state of Arkansas in 2012 to 2015. Observations have shown fields having a clay loam soil texture as more prone to the autumn decline phenomenon than others commonly cropped to rice. The root rotting symptoms often start a few weeks after flood establishment and become progressively worse throughout the season if unmitigated. In situations where root rotting is severe, fungi grow into the crown which limits function of the whole root system and prevents translocation of water and nutrients from the soil to the plant. In moderate to severe cases, tillers break off easily and plant death may occur rapidly leading to significant yield losses. Twenty commercial rice varieties were evaluated in the field in 2015 and based on their reactions a rating scale was developed. The nature of autumn decline problem, reactions of the varieties and the rating scale will be presented.

#### **Global Analysis of Horizontal Gene Transfer in *Fusarium verticillioides***

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The co-occurrence of microbes within plants and other specialized niches may facilitate horizontal gene transfer (HGT) affecting host-pathogen interactions. We recently identified fungal-to-fungal HGTs involving metabolic gene clusters. For a global analysis of HGTs in the maize pathogen *Fusarium verticillioides* (*Fv*), we used a phylogenomic pipeline to identify potential candidates. From an initial output of 1801 genes, manual curation revealed 117 strong HGT candidates acquired mostly from bacteria. Preliminary evaluation suggested several enriched metabolic pathways (e.g. lysine biosynthesis and nitrogen metabolism) compared to the frequency of such genes within the *Fv* genome. Interestingly, among the five candidates that were categorized as being involved in lysine biosynthesis, FVEG\_09873 appears only in *Fv* and has no orthologs in other fungi. This gene was acquired from plant-associated Proteobacteria and putatively encodes a diaminopimelate epimerase in the diaminopimelic acid pathway employed by most bacteria but not commonly found in fungi. Another promising HGT candidate acquired from bacteria is FVEG\_10494 that encodes a putative class-III aminotransferase, functioning in nitrogen metabolism. It is highly expressed when *Fv* is exposed to nitric oxide. Additional analyses will focus on other HGT candidates having limited fungal distribution. Functional characterization is underway to determine the significance of select HGT candidates in fungal adaptation.

#### **A Battle in a Kernel: Molecular Exploration of Antagonisms between Two Maize Endophytes, *Fusarium verticillioides* and *Acremonium zeae***

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*Fusarium verticillioides* (*Fv*) is a prevalent seed-borne maize endophyte capable of causing severe kernel rot and fumonisin mycotoxin contamination. Within maize kernels, *Fv* is primarily confined to the pedicel, while another seed-borne fungal endophyte, *Acremonium zeae* (*Az*), is observed in embryos. *Az* produces two lactam-containing antibiotics, named pyrrocidine A (PA) and B (PB), with PA exhibiting greater inhibitory activity against *Fv* and other fungi. PA differs from PB only by having a double bond in the lactam ring, suggesting the importance of the lactam moiety for toxicity. Preliminary experiments indicated the fungus is able to metabolize PB. In the *Fv* genome, 38 out of the 46 identified lactamase domain-containing proteins are predicted to carry out lactam hydrolysis. We recently characterized one lactamase gene (FVEG\_08291) essential for the hydrolysis of 2-benzoxazolinone, a maize phytoanticipin, that significantly induced the expression of this lactamase and adjacent clustered genes. Likewise, we predict that pyrrocidines will induce one or more lactamase gene(s) and that the encoded enzyme(s) will function to hydrolyze these *Az* metabolites. RNA-seq experiments are underway to assess the *Fv* transcriptional response to pyrrocidines and to *Az* co-inoculation. RNAseq data should allow us to identify the interacting lactamase gene(s) and help us better understand the molecular mechanisms underlying the metabolic antagonism between these endophytes.

#### **A dynamic regulatory network model underlying stomatal infection in *Cercospora zeae-maydis***

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*Cercospora zeae-maydis* causes gray leaf spot of maize, which is considered to be one of the most important foliar diseases of maize worldwide. Intriguingly, *C. zeae-maydis* has evolved an infection strategy that exploits host stomata. Prior to entering the host, the fungus undergoes a series of defined morphogenetic transitions collectively termed pre-infectious development. Despite the importance of pre-infectious development, the underlying genetics are poorly understood. Genetic regulatory network (GRN) modeling offers a novel approach to understand the complexity underlying plant-

pathogen interactions. The overarching goal of this project is to utilize GRN modeling to identify central regulatory hubs that control pre-infectious development in *C. zea-maydis*. To this end, we first developed a methodology to collect fungal tissue from inoculated maize during pre-infectious development and extract RNA of sufficient quality for RNA sequencing (RNA-seq). RNA-seq data is being generated via the Ion Torrent platform, and will be used to generate a GRN model. Preliminary transcriptome analysis has revealed that genes involved in transcriptional regulation, signaling, and stress and defense are enriched during pre-infectious development. Defining pre-infectious development at the systems level will provide mechanistic insight into the developmental program employed by *C. zea-maydis* and augment efforts to identify antifungal targets and improve disease resistance.

#### **Arabidopsis thaliana: A model host plant to study plant-pathogen interaction using rice false smut isolates of *Ustilagoidea vires***

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Rice false smut fungus which is a biotrophic fungal pathogen causes an important rice disease and brings a severe damage where rice is cultivated. We established a new fungal-plant pathosystem where *Ustilagoidea vires* was able to interact compatibly with the model plant *Arabidopsis thaliana*. Disease symptoms were apparent on the leaves of the plants after 6 days of post inoculation in the form of chlorosis. Cytological studies showed that *U. vires* caused a heavy infestation inside the cells of the chlorotic tissues. Development and colonization of aerial mycelia in association with floral organ, particularly on anther and stigma of the flowers after 3 weeks of post inoculation was evident which finally caused infection on the developing seeds and pod tissues. The fungus adopts a uniquely biotrophic infection strategy in roots and spreads without causing a loss of host cell viability. We have also demonstrated that *U. vires* isolates infect *Arabidopsis* and the plant subsequently activates different defense response mechanisms which are witnessed by the expression of pathogenesis-related genes, *PR-1*, *PR-2*, *PR-5*, *PDF1.1* and *PDF1.2*. The established *A. thaliana-U. vires* pathosystem will now permit various follow-up molecular genetics and gene expression experiments to be performed to identify the defense signals and responses that restrict fungal hyphae colonization in planta and also provide initial evidence for tissue-adapted fungal infection strategies.

#### **Macrophomina phaseolina promotes charcoal rot susceptibility in sorghum through induced host nitric oxide (NO) production**

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*Macrophomina phaseolina* (MP) is an important necrotrophic pathogen causing charcoal rot disease in sorghum (*Sorghum bicolor* (L.) Moench). Exploring the host-pathogen interaction is a key to understanding the molecular basis of resistance. An RNA-Seq experiment was conducted to discover differentially expressed genes (DEG) in response to MP infection. SC599 (resistant) and Tx7000 (susceptible) sorghum lines were grown in the greenhouse and inoculated with MP and phosphate-buffered saline (mock-inoculated control). RNA was extracted from three biological replicates at 2, 7, and 30 d post-inoculation (d.p.i.) from stem tissues adjacent to the inoculation point and subjected to RNA-Seq. Among the DEG, three genes (*Sb08g011530*, *Sb05g000680*, and *Sb04g027860*) involved in NO biosynthesis were significantly upregulated in Tx7000 at 7 d.p.i., while those of SC599 were not differentially expressed. To further confirm the findings, confocal microscopy was used. At 7 d.p.i., in situ NO production in MP- and mock-inoculated stem cross sections of two resistant (SC599, SC35) and two susceptible (Tx7000, BTx3042) lines was analyzed using 4-amino-5-methylamino-2',7'-difluorofluorescein diacetate (DAF-FM DA). Strong green fluorescence was observed in both MP-inoculated susceptible lines confirming the presence of NO while no signal was detected in the resistant lines. We believe that NO-mediated cell death contributes to the rapid proliferation of MP within susceptible hosts.

#### **Induced rhamnose biosynthesis in sorghum by *Macrophomina phaseolina* contributes to charcoal rot susceptibility**

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*Macrophomina phaseolina* (MP) is a necrotrophic pathogen that causes charcoal rot disease in sorghum (*Sorghum bicolor* (L.) Moench). To detect differentially expressed genes (DEG) in response to MP infection, an RNA-Seq experiment was conducted. SC599 (resistant) and Tx7000 (susceptible) sorghum lines were stalk-inoculated with MP hyphal fragments and phosphate-buffered saline (control). Stalk RNA extracted at 2, 7, and 30 d post-inoculation (d.p.i.) was subjected to RNA-Seq. Among the DEG, 7 genes involved in the rhamnose biosynthetic pathway (*Sb01g038050*, *Sb01g039220*, *Sb01g043370*, *Sb02g029130*, *Sb08g022850*, *Sb09g018070*, and *Sb10g024490*) were significantly upregulated in Tx7000 at 7 d.p.i., while those of SC599 showed no differential expression. To confirm the results, stem juice extracted from two MP- and mock-inoculated resistant (SC599, SC35) and susceptible (Tx7000, Btx3042) sorghum lines were subjected to HPLC. Compared to control, the MP-inoculated charcoal rot susceptible lines had significantly higher ( $P < 0.005$ ) stalk rhamnose concentrations (mg/mL) (Tx7000, 7.2; BTx3042, 5.8), while no such effect was evident in the resistant lines (SC599, 1.6; SC35, 1.4). To test the effect of rhamnose on MP growth, two isolates were grown on minimal medium supplemented with rhamnose at 0, 25, 50, 75, 100, and 125 mM concentrations. MP grew on rhamnose in a concentration-independent manner. Results suggested the potential role of host rhamnose as a virulence factor for MP.

#### **Macrophomina phaseolina induces stalk senescence in charcoal rot susceptible sorghum genotypes through up-regulated host chlorophyllase-2 biosynthesis**

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Charcoal rot (*Macrophomina phaseolina*, MP) and Fusarium stalk rot (*Fusarium thapsinum*, FT) are important sorghum diseases worldwide. An RNA-Seq experiment was conducted to explore the interaction of MP and FT with known resistant and susceptible sorghum genotypes. SC599 (R) and TX7000 (S) sorghum lines were grown in the greenhouse and inoculated with FT, MP, and phosphate-buffered saline (control). RNA extracted from stem tissues at 2, 7, and 30 days post-inoculation (d.p.i.) was subjected to RNA-Seq. At 7 d.p.i., a gene that encodes *chlorophyllase-2* (*Sb02g012300*) showed two and 200-fold up-regulation in FT- and MP-inoculated Tx7000, respectively. SC599 did not show a significant differential expression. To confirm the effect of up-regulated *chlorophyllase-2*, stalk chlorophyll was extracted with acetone from two pathogen- and mock-inoculated resistant (SC599, SC35) and susceptible (Tx7000, BTx3042) sorghum lines at 7 d.p.i. Total chlorophyll content (mg/g stalk) was computed using spectrophotometer readings. Compared to control, MP significantly reduced total chlorophyll of Tx7000 (control, 1.56; MP, 0.40) and BTx3042 (control, 1.45; MP, 0.63) ( $P < 0.016$ ). No such effect was evident with the two resistant genotypes. FT did not significantly affect the chlorophyll content of any genotype. Results revealed the ability of MP to induce stalk senescence through increased chlorophyll degradation, which contributes to the enhanced vulnerability to this necrotrophic fungus.

#### **Genome-wide study on cultivar-specific and stripe rust responsive miRNAs in *Triticum aestivum***

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Wheat represents approximately 30% of the world's grain production with more than 220 million cultivated hectares. Productivity at harvest is mainly governed by genetic composition and environmental factors influencing growth and development. Understanding the genetic factors that regulate these processes will help in developing varieties with better yield potential and disease resistance. MiRNAs are regulators of gene expression in eukaryotes that control myriad processes from development to abiotic and biotic stress responses. Using 12 small RNA libraries prepared from two wheat cultivars infected with stripe rust fungus (*Puccinia striiformis*), we identified 43 previously-known miRNAs, 50 novel variants of known miRNAs and 93



candidate novel miRNAs. Digital gene expression revealed 20 cultivar-specific miRNAs, 6 miRNAs differentially expressed between the two cultivars and 4 miRNAs responsive to stripe rust infection. These results were validated using RT-PCR and qRT-PCR. Using different target prediction algorithms, 69 wheat miRNAs were found to target fungal genes - a majority of which coded for small, secreted, cellular proteins. These results suggest a trans-kingdom regulation of gene expression potentially involved in the cultivar-specific resistance to stripe rust. Overall, this study contributes to the current repository of wheat miRNAs and provides novel information on the as yet uncharacterized roles for miRNAs in wheat host-pathogen interactions.

#### **Genotype and chemotype profiles of endophytes associated with wild barley**

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The genetic and chemotypic diversity of *Epichloë* species (Clavicipitaceae, Ascomycota) isolated from wild barley (*Hordeum* spp.) was explored to provide potential valuable resources for forage improvement. We screened 56 wild barley PI lines from the US Department of Agriculture's National Plant Germplasm System (USDA-NPGS), and identified 16 PI lines with endophyte incidence. Five distinct endophyte genotypes representing three nonhybrid and two hybrid *Epichloë* species were identified among the isolated endophytes from nine viable PI lines. Genotypes were identified based on the presence of alkaloid biosynthesis and mating type genes. Molecular phylogenetic analyses indicated that all nonhybrid genotypes are *E. bromicola*, whereas the two hybrid genotypes are *E. bromicola* by either *E. typhina* or an ancestor of *E. amarillans* or *E. baconii*. One of the hybrid genotypes was identified as a new taxonomic group currently referred to as HboTG-3. Alkaloid profiles were predicted from the genotyping data and the chemical profiles of distinct isolates were confirmed by HPLS and mass spectrometry with endophyte-infected plants, which confirmed the predicted chemotypes. Whole genome sequencing of four isolates provided insights for the complete alkaloid biosynthesis clusters in the wild barley endophytes and the unique features of the *Epichloë* species genomes. The beneficial effects each endophyte may provide the host will be tested under various biotic and abiotic stresses.

#### **Exploring the *Monilinia vaccinii-corymbosi*-blueberry pathosystem for the discovery of genes governing host-specificity**

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*Monilinia vaccinii-corymbosi*, a Sclerotiniaceae member, causes mummy berry disease on blueberries. Primary infection of vegetative tissues is caused by ascospores and secondary infection of flowers is caused by conidia. Fruit infection is highly specific as conidial germ tubes enter the host through flowers. Conidia germinate on stigma, traverse the stylar canal to colonize developing ovules, converting fruit into a mummy. To understand the underlying genetics of this specificity, we are developing standardized tools for generating inoculum, visualizing the course of infection, and collecting infected materials for transcriptomic analysis. A comparison of culture media and temperature conditions demonstrated that four day old cultures of *M. vaccinii-corymbosi* isolate RL1 (Mvc-RL1) grown on oatmeal agar medium, in the dark, at 22°C followed by a low temperature shift (15°C) induced sporulation most abundantly after 24 hours. For characterization of the gynoeceum infection process, a protoplast-mediated genetic transformation system was utilized to produce constitutive GFP-expressing Mvc-RL1 strains. Floral inoculation of Southern High Bush variety Legacy with conidia of GFP-Mvc-RL1 documented germinating conidia and the course of hyphae traversing the stylar canal. Using these tools, floral infections will be utilized for comparison with uninoculated flowers for transcriptomic analysis and identification of genes underlying host specificity in *M. vaccinii-corymbosi*.

#### **Fungal transcriptome analysis of the *Sclerotinia sclerotiorum* and *Pisum sativum* interaction**

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*Sclerotinia sclerotiorum* is a broad host range necrotrophic pathogen that causes white mold on pea (*Pisum sativum*). Gene expression profiling during infection has been characterized for *S. sclerotiorum* on several model plants. However, the *S. sclerotiorum*-*P. sativum* interaction is still poorly understood. In this study, fungal transcriptomic data was collected at three time points: 12 h, 24 h, and 48 h post inoculation (hpi), from susceptible and partially resistant pea cultivars inoculated with *S. sclerotiorum*. As a control, transcriptomic data was also collected from mycelia grown on PDA medium used in the inoculations. Four gene clusters were identified using expression profile analysis, one cluster was exclusively up-regulated on PDA, while the remaining three clusters were either associated with the host or sampling time. Gene ontology enrichment analysis demonstrated that 83% of the up-regulated cluster 1 genes were categorized as oxidoreductases at 12 hpi. Whereas, 43% of the up-regulated cluster 2 genes were involved in hydrolysis and transport functions at 24 and 48 hpi. The temporal gene expression profile change may indicate the lifestyle transition of the pathogen from biotroph to necrotroph. In addition, three out of 486 predicted effector candidate genes were up-regulated *in planta* at 24 and 48 hpi. The identification of key genes involved in the *S. sclerotiorum*-*P. sativum* interaction will facilitate effector assisted breeding for disease management.

#### **Aspartyl protease mediated cleavage of AtBAG6 triggers autophagy and fungal resistance in plants**

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The Bcl-2-associated-athanogene (BAG) family is an evolutionarily conserved group of co-chaperones that modulate numerous cellular processes. Previously we found that *Arabidopsis* BAG6 (AtBAG6) is required for basal immunity against the fungal phytopathogen *Botrytis cinerea*. However, the mechanism(s) by which AtBAG6 controls immunity are obscure. Here, we address this important question by determining the molecular mechanisms responsible for AtBAG6 mediated basal resistance. We show that AtBAG6 is cleaved *in vivo* in a caspase-1 like dependent manner, and via a combination of pull-downs, mass spectrometry, yeast-two-hybrid and chemical genomics, we demonstrate that AtBAG6 interacts with a C2-GRAM domain protein (AtBAGP1) and an aspartyl protease (AtAPCB1), both of which are required for AtBAG6 processing. Furthermore, fluorescence and transmission electron microscopy established that AtBAG6 cleavage triggers autophagy in the host that coincides with disease resistance. Targeted inactivation of *AtBAGP1* or *AtAPCB1* results in the blocking of AtBAG6 processing and loss of resistance. Mutation of the cleavage site blocks cleavage, inhibits autophagy and disease resistance is compromised. Taken together, these results couple an aspartyl protease with a molecular co-chaperone to trigger autophagy and plant defense, providing a key link between fungal recognition and the induction of cell death and resistance.

#### **The MAP kinase kinase kinase gene, *CpBck1*, regulates sectorization and pathogenicity of the phytopathogenic fungus *Cryphonectria parasitica***

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*CpBck1*, an ortholog of the cell-wall integrity mitogen-activated protein kinase kinase kinase of *Saccharomyces cerevisiae*, was cloned and characterized from the chestnut blight fungus *Cryphonectria parasitica*. The *CpBck1*-null mutant displayed cell wall integrity-related phenotypic changes such as abnormal cell morphology and wall formation and hypersensitivity to cell wall-disrupting agents. In addition, the mutant showed severely retarded growth without any sign of normal development, such as hyphal differentiation, conidiation, or pigmentation. As the culture proceeded, the mutant colony showed sporadic sectorization. Once sectored, the sectored phenotype of robust mycelial growth without differentiation was stably inherited. Compared with the wild type, both the parental *CpBck1*-null mutant and the sectored progeny exhibited marked impaired virulence. The present study

revealed that a mutation in a signaling pathway component related to cell-wall integrity resulted in sporadic sectorization and these sectorized phenotypes were stably inherited, suggesting that this signal transduction pathway is implicated in adaptive genetic changes for sectorization.

#### **A band of misfits: Role of unexpected proteins in the plant symbiotic signaling pathway**

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Rhizobia and arbuscular mycorrhizal fungi produce lipo-chito-oligosaccharidic signals that are perceived by legume hosts at the plasma membrane level. These signals are then transduced to the nucleus where they trigger oscillations of the nuclear calcium concentration (calcium spiking) that, in turn, regulates symbiotic gene expression. Genetic and protein interaction studies identified some relatively expected components in such a signaling pathway: receptor-like kinases at the plasma membrane that perceive the microbial signals, ion channels and ion pumps on the nuclear envelope that allow calcium spiking, and nuclear transcription factors that regulate gene expression. We will present new data on more unexpected members of this signaling pathway. (1) An HMGR (3-Hydroxy-3-Methylglutaryl Coenzyme A Reductase) that interacts with and is regulated by the symbiotic receptor kinases. In particular, we will show that the activity of this HMGR is not only necessary but also sufficient to trigger calcium spiking. (2) Nucleoporins of the NUP107-160 sub-complex that have been identified through forward genetic approaches. We will show that these nucleoporins are required for the proper localization of symbiotic ion channels to the inner nuclear membrane. Finally, we will compare results between two legumes, *Medicago truncatula* and *Lotus japonicus*, and show the value of comparing carefully these two genetic systems.

#### **Functional analysis of root-knot nematode (*Meloidogyne javanica*) virulence genes in rice**

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Root-knot nematodes are endo-parasites with a wide host range, encompassing mono- and dicotyledonous plant crops. *Meloidogyne javanica* is responsible for rice (*Oryza sativa*) production losses in Brazil and Africa (Benin). Successful infection is likely achieved by effector proteins produced in the nematode esophageal gland cells and released in the host plant cells. The aim of this study was to assess the functional role of three esophageal gland cell proteins of *M. javanica* in rice - nematode interactions. RT-qPCR assays showed that *Mj-SP2*, *Mj-SP18* and *Mj-SP19* genes are over-expressed all along the infection cycle in rice roots. Immunodetection experiments using specific antibodies raised against synthetic peptides showed that the nematode proteins are localized in circular granules structures within the nematode body. Transgenic rice (*O. sativa Nipponbare*) plants expressing the candidate proteins or artificial micro-RNAs (amiRNAs) able to silence the cognate genes in the nematode were produced. Assessment of nematode reproduction on homozygous transgenic lines allowed the identification of rice lineages with altered susceptibility, indicating that these proteins may be involved in *M. javanica* virulence. Data obtained significantly widen our knowledge of molecular players contributing to nematode pathogenicity, and open new avenues for nematode control strategies in rice and other crops of interest.

#### **MetacodeR: An R package for comparative analysis and visualization of microbial communities and evaluation of metabarcoding primer specificity**

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Metabarcoding, the sequencing of a locus amplified from environmental DNA, is revealing the unculturable majority of microbes for the first time, but new methods present new challenges. Complex hierarchical data are difficult to plot and the commonly used stacked bar charts are ineffective when plotting multiple taxonomic ranks or treatments. In addition, the choices of loci and barcode primers are a source of underexplored bias. MetacodeR is a new tool kit for metabarcoding and community ecology, focusing on visualization of taxonomic data and *in silico* primer/barcode evaluation. It provides tools to integrate quantitative data from thousands of taxa and multiple communities into intuitive tree-based plots. Data from one or more communities are mapped to tree element size or color, as in a heat map. This allows for rapid exploration of data and information-rich, publication-quality graphics. In addition, metacodeR provides critical tools for evaluating potential primers and barcode loci. *In silico* PCR evaluates primer specificity and barcode gap analysis estimates error rates and resolution. MetacodeR is currently being applied to evaluating the diversity of fungi and oomycetes in the rhizosphere of nursery-grown rhododendron. The ability to easily test new primers/barcodes will accelerate the adoption of metabarcoding to emerging or understudied pathogen groups, such as oomycetes, and intuitive plotting will make subtle patterns in complex data more apparent.

#### **Molecular dissection of resistance signaling in watermelon fruit through transcriptomic approach**

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Plant defense signaling involves a complex array of events initiated by direct or indirect interactions between pathogen effectors and host resistance proteins. Our study involves developing watermelon (*Citrullus lanatus*) lines resistant to various pests and pathogens through conventional breeding techniques. One of the most common and widely spread diseases in watermelon is Phytophthora fruit rot caused by *Phytophthora capsici*, which is especially severe in the southeastern US due to prevalence of favorable weather conditions. In order to elucidate the molecular mechanism of signaling events that occur during watermelon - *Phytophthora* interactions we are utilizing the available watermelon and *P. capsici* genome sequence data along with our RNA-seq data to identify the differentially regulated transcriptome between resistant and susceptible watermelon lines. Our preliminary data indicates that most sample sequence reads matched to watermelon genome (70-90%) and the pathogen transcript reads were higher in susceptible cultivar 'Sugar Baby' (5-30%) when compared to resistant germplasm line 'USVLO20-PFR' (0.5-1%). The genotypic data correlates well with the phenotypic data. The overall goal is to identify plant defense markers contributing to host resistance against *P. capsici* that will prove useful in watermelon breeding programs.

#### **Identification of silencing suppressor proteins in Maize chlorotic mottle virus**

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Maize Lethal Necrosis (MLN) is a devastating disease of maize caused by a synergistic interaction between *Maize chlorotic mottle virus* (MCMV) and *Sugarcane mosaic virus* (SCMV), *Wheat streak mosaic virus* (WSMV) or *Triticum mosaic virus* (TriMV). RNA silencing is a prominent antiviral

defense system in plants. In single and double infections, MCMV and SCMV activate maize antiviral RNA silencing machinery, resulting in the accumulation of virus-derived small RNAs. Most plant viruses encode silencing suppressor proteins to inactivate RNA silencing and overcome the host defense system. No silencing suppressor has been identified in MCMV. To identify silencing suppressors in MCMV, individual proteins were cloned into binary vectors for transient expression in *Nicotiana benthamiana*. Proteins with silencing suppression activity were identified by the ability to complement the pathogenicity defect in two different suppressor-deficient viruses. Identification and characterization of silencing in MCMV and SCMV establishes the foundation to further study the molecular mechanisms involved in Maize Lethal Necrosis Disease.

#### **Resistance of cultivated soybean to *Clover yellow vein virus* apparently originated during domestication from wild soybean**

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*Clover yellow vein virus* (CIYVV) (genus *Potyvirus*, family *Potyviridae*), is pathogenic to legume plants, including broad bean (*Vicia faba*) and pea (*Pisum sativum*). However, inoculation of dozen cultivated soybean (*Glycine max*) genotypes with CIYVV strain no30 (CIYVV-no30) did not result in systemic infection in any of the inoculated plants. In contrast, inoculation of 24 accession lines of wild soybean (*G. soja*) with the green fluorescent protein (GFP)-tagged CIYVV-no30 (CIYVV-no30-GFP) resulted in systemic infection in all, except one line. This observation led us to hypothesize that resistance to CIYVV in cultivated soybean originated from wild soybean during domestication. It seems likely that during soybean domestication, parental plants with natural resistance to CIYVV were selected. To test this hypothesis, and to elucidate the genetic basis of the resistance, recombinant inbred lines (RILs) were generated from a cross between cultivated soybean TK780 and wild soybean line B01167. Analysis of responses of 64 derivative RILs inoculated with CIYVV-no30-GFP resulted in identification of one major and one minor quantitative trait loci (QTL). The role of each of these loci in conferring resistance to CIYVV in cultivated soybean is being investigated.

#### **Examination of the mechanism of superinfection exclusion by *Citrus tristeza virus***

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Superinfection exclusion (SIE), referred to as cross-protection when used as an agricultural practice, is a phenomenon in which a primary virus infection prevents a secondary infection with the same or closely related virus. Earlier we demonstrated that SIE by *Citrus tristeza virus* requires viral p33 protein, yet p33 alone is not sufficient for virus exclusion. In this study we show that a 5'-proximal genomic region encoding leader proteases L1 and L2 is involved in SIE. Substitution of this region with the cognate sequences from a heterologous strain affected virus ability to exclude the parental virus. As the next step, we are interested to assess whether SIE is conferred by the L1/L2 proteins or by their coding sequences. We created four mutant viruses having alterations in the RNA sequences of the L1 and L2 N-terminal (NTD) and protease domains by changing the wobble base of each possible codon to avoid changing the amino acid sequences of these proteins. All mutant viruses were able to replicate in *Nicotiana benthamiana* at the levels comparable to the wild type virus, and three of them except L1 NTD mutant were able to establish systemic infection in *N. benthamiana* and citrus plants. Additional constructs containing mutations within the L1 NTD domain are being made with the goal to produce virus variants capable of systemic infection of both plant hosts. The effect of the introduced mutations in the L1-L2 domains on SIE ability will be assessed.

#### **Exchange of HC-Pro cistron between *Soybean mosaic virus* and *Clover yellow vein virus*: Impact on pathogenicity**

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*Soybean mosaic virus* (SMV)-N and *Clover yellow vein virus* (CIYVV)-no30, members of *Potyvirus* genus within *Potyviridae* family, have narrow host range. SMV-N is well adapted to cultivated soybean (*Glycine max*) while infects systemically wild-type soybean (*G. Soja*) as well. However, SMV-N is incapable of systemic infection in broad bean (*Vicia faba*). In contrast, CIYVV-no30 is capable of systemic infection in both broad bean and wild-type soybean; however, incapable of systemic movement in cultivated soybean. Because SMV-N and CIYVV-no30 both establish local infection in all these plants, we hypothesized that lack of systemic movement by SMV-N in broad bean and by CIYVV-no30 in cultivated soybean is due to incompatibility of respective HC-Pro, a key long-distance movement potyviral protein, in these two hosts. To test this hypothesis, chimeras were constructed with precise exchanges of HC-Pro cistrons between SMV-N and CIYVV-no30 and inoculated onto cultivated soybean "Williams", wild-type soybean "B01167" and broad bean "Windsor". Analyses showed that pathogenicity of recombinant viruses did not differ from those of the parental viruses. This observation suggests that i) HC-Pro of SMV-N and CIYVV-no30 are functionally equivalent, and ii) the determinant(s) for systemic infection in cultivated soybean by SMV-N and by CIYVV-no30 in broad bean reside elsewhere.

#### **Devising a custom 13K unigene-based *Nicotiana benthamiana* microarray to study host-pathogen interactions at the transcriptome level**

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Selecting a model plant to study host/pathogen interactions at the transcriptome level is a crucial part of experimental design. Although *Arabidopsis thaliana* is a preferred model based on the availability of comprehensive genomic information, it is only susceptible to a restricted number of pathogens. Due to this limitation, exploring alternative plant models is necessary. *Nicotiana benthamiana* (Nb) is highly susceptible to a wide range of pathogens. In order to enable the use of Nb in high-throughput studies, we created a genomic tool for this plant. We developed the first Nb array from 13,014 unigenes (average 752 bp) which were originally derived from 40,000 expressed sequence tags (ESTs; ~70% of publicly available Nb ESTs representing ~38% of the transcriptome). Homology searches against GenBank, KEGG, and GO databases (BLASTX,  $E$ -value  $< 1 \times 10^{-5}$ ) indicated that 66% of the unigenes have > one hit, with the most frequent hit to *Solanaceae* plants. Our unigene-based array has broad coverage of plant pathways: metabolism, transportation, transcription, translation, signal transduction and defense. 261 unigenes were involved in transcription with the most abundant ones belonging to the AP2/ERF, WRKY and GRAS families. The Nb array consists of 9 probes per unigene. We conducted an array performance test to study interactions between Nb and *Red clover necrotic mosaic virus*. The array successfully yielded 100% hybridization capacity to Nb mock and infected samples.

#### **Reimagining the *Cauliflower mosaic virus* genome as an interactome between host and virus proteins**

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The genome of *Cauliflower mosaic virus* (CaMV) was the first plant-associated organism to be completely sequenced, in 1980. The CaMV nucleotide sequence revealed that its genome was composed of seven substantial open reading frames (ORFs), and six proteins were subsequently matched up with their respective ORFs; the seventh putative protein has never been found in CaMV-infected plants. Since the initial CaMV sequence was finalized, numerous studies have been completed to ascertain the functions of the six CaMV proteins, as well as their subcellular localization. Furthermore, many of the CaMV proteins have been the subject of yeast two-hybrid and co-immunoprecipitation screens to characterize the interactions of host and virus proteins. For example, the P6 protein of CaMV is a multifunctional protein with distinct roles in translation, intracellular movement, elicitation of defenses in resistant hosts or symptoms in susceptible hosts, and modulation of plant defenses. The P6 protein has been shown to interact with at least 14

host and virus proteins. This presentation will illustrate how interactome maps coupled with subcellular localization studies can be used to describe the CaMV disease cycle.

### **Two eIF4E proteins in Yellow Lantern chili pepper interact with *Pepper veinal mottle virus* VPg**

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*Pepper veinal mottle virus* (PVMV) causes a major disease on the Yellow Lantern chili peppers (*Capsicum chinense*), an economically important specialty crop on Hainan Island, China. PVMV is a *Potyvirus* with a genome-linked protein (VPg) attached at the 5' end of the (+)-strand RNA genome. Interaction between PVMV VPg and pepper eIF4E is necessary for the PVMV to infect peppers. As there is no natural resistance against PVMV in the Yellow Lantern chili pepper, disruption of the VPg-eIF4E interaction by genome editing appears to be a feasible approach to engineer PVMV resistance. Four eIF4E genes were identified from bioinformatic analysis of pepper genomes, cloned by RT-PCR with specific primers, and analyzed by sequencing. The pepper eIF4E family consists of two eIF4E genes (Cc-eIF4E and Cc-eIF4E-Xm), one eIF(iso)4E gene (Cc-eIF(iso)4E), and one novel cap binding protein gene (Cc-nCPB9090). Only Cc-eIF4E and Cc-eIF(iso)4E interacted with PVMV VPg in the LexA yeast two-hybrid system. The specific interaction between these two pepper eIF4E proteins and PVMV VPg was further studied *in vivo* with bimolecular fluorescence complementation (BiFC) tests in infiltrated tobacco cells. Cc-eIF4E showed a strong and consistent interaction while Cc-eIF(iso)4E displayed a weak and inconsistent interaction with PVMV VPg by BiFC. These two eIF4E genes will be further evaluated as potential targets for genome editing in our quest for PVMV resistance in the chili pepper.

### **QTL Analysis of flg22-triggered Basal Resistance in Maize**

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Plants are constantly under attack by different pathogens. Molecules released or generated during microbial entry can be perceived by the plant and produce activate defense, including early responses such as the oxidative burst. An important component of this defense system is based upon the recognition of microbe-associated molecular patterns (MAMPs also known as pathogen-associated molecular patterns or PAMPs). Once MAMPs are detected by the plant, a defense response, termed MAMP-triggered immunity (MTI, also known as PAMP-triggered immunity or PTI) happens. In a few cases, a direct link has been established between MTI and specific quantitative trait loci (QTLs) for partial resistance. The MTI response has been widely studied, mainly in the model plant *Arabidopsis* and a few crops, such as Brassica and soybean. Few studies have examined MTI in maize. There is significant potential in harnessing maize MTI to improve cultivars to withstand a variety of pathogens. In this study, we evaluated the MTI responses in 26 different maize genotypes by challenging with one well known MAMP (flg22), and then assessed a recombinant inbred line mapping population (CML228×B73) derived from parent lines with differential flg22 responses. We identified one major QTL on chromosome 2 associated with variation in the flg22 MAMP response. We will describe our progress in characterizing this QTL and in identifying QTL associated with other MAMP responses.

### **Physiological and Molecular-Genetic Characterization of Basal Resistance in Sorghum**

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Early detection of pathogens is a critical component of plant immunity. Basal resistance, aka innate immunity, is the first line of defense to protect plants against a range of pathogens. Triggered by the recognition of microbe-associated molecular patterns (MAMPs) by pathogen recognition receptors (PRRs), the induction of basal resistance has been shown to vary quantitatively within and across species. In sorghum (*Sorghum bicolor* (L.) Moench), preliminary results supports this hypothesis. The goal of this project is to investigate the genetic architecture and transcriptional response associated with the basal defense response in sorghum. Specific objectives in this project are to 1. Develop robust assays to measure disease resistance and the MAMP response in sorghum, and screen a set of diverse sorghum germplasm for variation in these traits, 2. Identify genes differentially regulated during the MAMP response in high and low responding sorghum genotypes, and 3. Examine the activity of fungal extracts of sorghum pathogens in inducing the MAMP response, 4. Measure the effect of the MAMP response on disease resistance in controlled assays, and 5. Determine whether control of variation in the MAMP response and variation in disease resistance is under shared genetic control.

### **The Role of Ethylene in PAMP-Triggered Immunity in *Fusarium* Crown Rot (FCR) Disease Resistance in Wheat**

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*Fusarium* Crown Rot (FCR) is a devastating cereal crop disease cause by necrotrophic fungal pathogens *Fusarium graminearum* and *Fusarium pseudograminearum*. Plants detect conserved pathogen-associated molecular patterns (PAMPs) through pattern-recognition receptors. Then, a signal cascade is triggered resulting in PAMP-Triggered Immunity (PTI) response such as expression of defense response genes and production of plant hormones jasmonic acid, salicylic acid, and ethylene. A model proposed by Zipfel in 2013 suggests that ethylene functions to prime additional signaling pathways for sustained PTI response. Our laboratory has shown that disease resistance to FCR can be enhanced in susceptible wheat cultivars by stimulating the ethylene-signaling pathway with ethylene biosynthetic precursor 1-aminocyclopropane-1-carboxylic acid (ACC). Additionally, resistant cultivars become more susceptible when ethylene-signaling is inhibited by treatment with the competitive inhibitor 1-methylcyclopropane (1-MCP). RNA-Seq analysis of FCR-susceptible wheat cv 'Kennedy' in response to ACC or 1-MCP application and *F. graminearum* inoculation demonstrates significant difference in Differentially Expressed Genes (DEGs) within several disease-response categories such as peroxidases, pathogenesis-related proteins, and WRKY transcription factors. Currently, we are examining the transcriptome of FCR-resistant cv 'Bobwhite' to identify additional candidate genes involved in FCR resistance.

### **Use of synchrotron Fourier transform spectroscopy to shed light on the chemical composition of cell wall appositions papillae on winter wheat leaf**

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Papillae formation is known as one of the most important plant immunity mechanisms. Its major function is to prevent the entry of fungal pathogens into the host plant cells. In this study, a synchrotron based Fourier transform spectroscopy (sFTIR) was used to elucidate the chemical composition of the cell wall of forming papillae and non-forming papillae in response to powdery mildew infection. Spectroscopic results indicated an increase in peaks intensity of asymmetric and symmetric stretching vibrations of CH<sub>3</sub> and CH<sub>2</sub> that correspond to fatty acid groups, phenolics groups, lignification, cellulose, hemicellulose, and glucans following pathogenic infection. Principal Component Analysis (PCA) applied to sFTIR spectra confirmed these findings and distinguished two different groups with significant differences in cell wall composition including lignin, phenolics, polysaccharides and carbohydrates. This study highlighted, for the first time, the potential application of synchrotron-based infrared technique to study the *in situ* composition of lignified papillae and plant cell in the plant disease resistance, which remained to be a challenge with biochemical approaches. Subsequently, further

studies with more cultivars, as well as other synchrotron imaging techniques will be undertaken to confirm these preliminary findings and explore other defense response mechanisms.

#### **miR858 functions as a negative regulator of plant susceptibility to the beet cyst nematode *Heterodera schachtii***

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Recent studies have indicated that microRNA genes play key roles in shaping the compatibility of the interaction between plant-parasitic cyst nematodes and host plants. In this study, we report a novel regulatory role of the Arabidopsis miR858 and its MYB83 transcription factor during plant-cyst nematode interactions. Promoter-GUS fusion assays pointed to a role of miR858 in post-transcriptional regulation of MYB83 in the syncytium induced by the beet cyst nematode *Heterodera schachtii*. qPCR quantification further supported the negative association between miR858 and MYB83 expression levels at different time points post *H. schachtii* infection. Overexpression of miR858 in Arabidopsis resulted in significant decreases of plant susceptibility to infection by *H. schachtii*, whereas a *mir858* mutant showed the opposite effect of increased susceptibility. In addition, we found that T-DNA insertional mutation of MYB83 was less susceptible to nematode infection. In contrast, overexpression of miR858-resistant *myb83* produced hyper-susceptibility phenotypes. Taken together, these data revealed the importance of tight regulation of MYB83 transcription factor expression by miR858 in the syncytium, apparently to control downstream target genes that encode components that may be needed for syncytium formation/function. Identification of target genes that are directly or indirectly regulated by MYB83 is currently underway.

#### **Agroinfiltration-based screening to discover new potato germplasm with resistance against *Globodera* nematode pests**

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Potato cyst nematodes (PCN) (*Globodera rostochiensis* and *G. pallida*) are of worldwide regulatory concern and considered to be the most economically important pests of potato, causing > 80% yield loss. The U.S. potato industry has been adversely affected due to the emergence of *G. pallida* in Idaho and a virulent *G. rostochiensis* pathotype in New York and the lack of resistant potato cultivars. Using host resistance is the most effective and sustainable means for nematode control. Like other plant pathogens, nematodes secrete effector proteins into root cells to suppress host plant defenses, leading to successful infection. However, specific effectors can be recognized by host resistance proteins and trigger plant defense, which often results in a hypersensitive response (HR) believed to halt pathogen infection. "Effectormics" is a high-throughput, functional genomics approach that uses effectors to potentially detect R genes in plants. Wild potato species offer an extremely rich source of resistance against *Globodera* nematodes. We have established an agroinfiltration assay in wild potato species. Our initial screening of forty-two wild potato accessions with a group of PCN-secreted effectors showed the occurrence of an HR phenotype when specific effectors were transiently expressed in potato leaves. The results indicate that this "Effectormics" approach may be a valuable tool that helps to accelerate resistance breeding against *Globodera* nematode pests.

#### **Transcriptome analysis of resistance against *Phytophthora cinnamomi* and the role of $\beta$ -cinnamomin elicitors in pathogenicity**

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Resistance to the destructive oomycete pathogen *Phytophthora cinnamomi* is rare. We have explored resistance in roots, following infection, of the Australian native monocot *Lomandra longifolia*. Inoculated plants developed restricted lesions and analysis of lignin, callose and hydrogen peroxide indicated their involvement in resistance. We then used a transcriptomic approach to further examine resistance in this system. As the genome of *L. longifolia* is not sequenced, we performed RNA-seq analysis and the data showed the elevated expression of a range of resistance-related genes including those involved in jasmonic acid synthesis, lignin biosynthesis and in disease resistance signalling. Blast2GO analysis of contigs revealed the presence of elicitor and elicitor-like proteins. We then investigated the role of elicitors in pathogenicity of *P. cinnamomi*. Elicitors were isolated from liquid culture and different isoforms were identified by isoelectric focusing analysis. A  $\beta$ -cinnamomin elicitor was purified, an antibody produced and its specificity was shown by Western blot analysis.  $\beta$ -cinnamomin production at different life stages of the pathogen was confirmed by confocal microscopy. Elicitors were also detected in inoculated roots of the susceptible plant species, *Lupinus angustifolius*. Elucidation of resistance-related mechanisms and pathogenicity factors provides opportunities for modification of susceptible plants and identification of targets to prevent infection.

#### **A synergistic effect of two plant antimicrobial peptides from defensin and lipid-transfer protein families towards *Phytophthora infestans***

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Late blight caused by *Phytophthora infestans* (Mont.) de Bary is one of the most important diseases afflicting potatoes and tomatoes worldwide. It results in economically meaningful yield losses and decreases in the quality of harvested produce. In the last two decades, researchers have focused on developing chemical and biological pesticides to treat against the diseases following plant tissue necrotization and dying off. Antimicrobial peptides are one of the primary components of a plant innate immunity to biotic stress factors involving diseases and pests. Most of these peptides can act directly against plant pathogenic microorganisms (fungi, oomycetes and bacteria) based on disruption of cell wall and plasma membrane integrity. In our study, we investigated two antimicrobial peptides (plant defensin Nsd2 isolated from *Nigella sativa* seeds and lipid-transfer protein EcLTP purified from *Echinochloa crusgalli* kernels) belonging to different plant defense families. We showed that these peptides exhibited synergistic action against *Phytophthora infestans* on potato tuber disks assays when used in tandem. An application of plant antimicrobial peptides that demonstrates a diverse mode of action towards *Phytophthora infestans* would enable increases in the inhibition effect up to complete elimination of the pathogen.

#### **Wheat streak mosaic virus P1: Defining the minimal region required for the suppression of RNA silencing**

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Wheat streak mosaic virus (WSMV) is the most economically important wheat virus in the Great Plains region of the USA. WSMV is the type species of the genus *Tritimovirus*, family Potyviridae, and is transmitted by the wheat curl mite, *Aceria tosichella* Keifer. Previously, we reported that WSMV P1 functions as the viral suppressor of RNA silencing (VSR) and pathogenicity enhancer. In this study, we examined the minimal region of P1 required for VSR function. Twenty-one deletion mutants of WSMV P1 were generated by progressively deleting from the N- and C-terminal regions. VSR assays were conducted by agro-infiltration of deletion mutants in pCASS4 together with the 35S-GFP into *Nicotiana benthamiana* leaves. WSMV P1 with deletions comprising up to 23 amino acids at the N-terminal region exhibited VSR activity, while deletion of N-terminal 25 amino acids completely abolished the VSR activity. In contrast, even a single amino acid at the C-terminus is indispensable for VSR activity of P1, suggesting that the C-terminal region of WSMV P1 is crucial for VSR activity. Mechanistic studies of WSMV P1 in VSR activity will be discussed.

#### **Development of HRM markers tightly linked to extreme resistance to *Potato virus Y* using next-generation sequencing**

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Tetraploid potato breeding clone F87084 possesses a resistance gene (*Ry*) that confers extreme resistance (ER) to *Potato virus Y* (PVY). However, markers reported to be highly linked to different *Ry* genes were not detected in F87084. Next generation sequencing of genomic DNA from F87084 (*Rrrr*), F02010 (*rrrr*) and the respective bulk DNA of resistant and susceptible progenies of F87084 x F02010 was carried out by Illumina HiSeq for identification of single nucleotide polymorphisms (SNP). Polymorphic positions matching the expected pattern in parents and progeny bulk samples were determined. The genomic regions flanking the target SNP were extracted and used to design primers and probes for high-resolution DNA melting (HRM) assays. Of the 10 primer/probe sets designed and tested, five produced amplicons that exhibit different HRM profiles between the resistant parent/bulk and the susceptible parent/bulk. Further analysis of the HRM primer/probe sets on a segregating population comprised of 300 progenies revealed near-perfect matches between the HRM assays and the phenotyping results, demonstrating high linkage between these newly developed HRM markers and *Ry* in F87084. The efficacy of the HRM markers for marker-assisted selection of ER to PVY is validated with different populations involving F87084.

#### **The virulence function and regulation of the metalloprotease gene *prtA* in the bacterial plant pathogen, *Burkholderia glumae***

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Bacterial panicle blight caused by *Burkholderia glumae* is a major bacterial disease of rice in the United States. Our preliminary RNA-seq study showed that a serine metalloprotease gene, named *prtA*, is positively regulated by the ToF1/ToFR-mediated quorum sensing system like other genes for producing important virulence factors of *B. glumae*, such as toxoflavin. To study the function of *prtA* in *B. glumae* 336gr-1, a null mutant of *prtA* was generated through an insertional mutagenesis approach. Extracellular protease activity was not detected when the *prtA* mutant was grown on LB-agar plates amended with 1% skim milk, indicating that *prtA* is solely responsible for the extracellular protease activity of *B. glumae* 336gr-1. In addition, inoculation of rice panicles with the *prtA* mutant resulted in reduction of disease severity compared to the wild type strain, showing the requirement of *prtA* for full virulence. This study also revealed that the regulation pattern of extracellular protease activity is similar to that of toxoflavin production. Particularly, it was newly found that *qsmR*, known as the quorum-sensing master regulator gene, is essential for both extracellular protease activity and toxoflavin production by *B. glumae* 336gr-1. Furthermore, additional genetic elements that control extracellular protease activity were newly identified through screening of random mini-Tn5 mutants, which provide useful information to understand the mechanism of *prtA* regulation.

#### **The genome of the endophytic *Curtobacterium* strain ER 1.4/2 and its potential as a biocontrol of several plant diseases**

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*Curtobacterium* are Gram-positive bacteria that can live inside of plants as endophytes and also have the ability to cause disease such as wilting disease in dry beans. *Curtobacterium* sp. strain ER1.4/2 (ER1.4/2) was isolated from branches of healthy sweet-orange (*Citrus sinensis*). The genomic DNA of this bacterium was extracted and the whole DNA sequencing was performed using next-generation DNA sequencing by the Illumina Hiseq 2000 method. The genome annotation of *Curtobacterium* strain ER1.4/2 was initially performed using a RAST (Rapid Annotation using Subsystem Technology) server. The draft genome contains 3,692,034 bases with a GC content of 71.6%. Important genes such as a bacteriocin and siderophores were found in the genome. This bacteria showed antibiosis *in vitro* against several important phytopathogens such as *Xylella fastidiosa* CVC, *Xanthomonas campestris* pv. *Glycines*; *Pseudomonas syringae* pv. *syringae* and *Agrobacterium tumefaciens*. We are currently identifying ER1.4/2 at the species level by FAME to confirm its 16S rDNA identification. In addition, studies on its interaction with several cultivars of *Phaseolus vulgaris* and *Glycine max* are underway. The ability of ER1.4/2 to colonize multiple plants, with its wide-spectrum antibiosis and non-pathogenicity to all plants tested make this bacterium an excellent biocontrol candidate against bacterial diseases.

#### **The *dlt* locus contributes to virulence in the necrotrophic pathogen *Pectobacterium carotovorum***

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In Gram-positive bacteria, the *dlt* locus contributes to antimicrobial peptide resistance through D-alanylation of cell wall teichoic acids. Homologs of *dlt* are present in the Gram-negative, soft rot pathogens *Pectobacterium* and *Dickeya*. Gram-negative bacteria lack teichoic acids, so the function of the *dlt* operon is unclear. We mutated the *Pectobacterium carotovorum dltB* and *dltD* genes and assayed for virulence attenuation in *Solanum tuberosum* (potato) stems and tubers. Red Norland plant stems were stab-inoculated and evaluated with a rating scale for wilt and stem maceration symptoms. Stem colonization by the different strains was determined by slicing and plating 1cm stem sections. Russet tubers were inoculated with serial dilutions of bacteria, and evaluated for the presence or absence of maceration. None of the mutants assayed were reduced in ability to cause symptoms on stems. However,  $\Delta dltD$  was reduced in ability to spread inside stems. In tubers, both mutants were reduced in virulence compared to the wild type strain. Therefore, the *dlt* appears to contribute to *P. carotovorum* virulence in tubers and spread in stems. Investigating possible targets of gene products using HPLC and biolig analysis are on-going and will provide insight into *dlt* function in *P. carotovorum*.

#### **Systematic computational network-based analysis to predict subnetwork modules associated with pathogenicity and fumonisins in *Fusarium verticillioides***

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*Fusarium verticillioides* is a notorious pathogen of maize causing ear rot and fumonisin contamination. In order to develop innovative preharvest control strategies, such as new biocontrol agents and resistant hybrids, to minimize the entry of fumonisins into our food supply, there is a need to identify new molecular targets for disrupting *F. verticillioides* pathogenicity. In this study, we performed a systematic computational network-based comparative analysis of two distinct *F. verticillioides* – maize kernel RNA-seq datasets (one on moderately resistant inbred and the other on susceptible hybrid). For the systematic analysis of the pathogenicity mechanism, we first inferred *F. verticillioides* co-expression networks. Subsequently, we identified functional subnetwork modules on the co-expression networks consisting of interacting genes that display harmoniously coordinated behavior in the respective datasets. A computationally efficient branch-out technique applied with an adopted probabilistic pathway activity inference method was used to identify functional subnetwork modules likely involved in *F. verticillioides* pathogenicity. Here we exhibit key potential subnetwork modules, where the modules contain several enriched GO terms as well as potential pathogenicity genes from other pathogenic fungi. Putative hub genes in each subnetwork will be functionally characterized to test their candidacy as new targets for ear rot and fumonisin control strategies.

#### **Computational and functional analyses of MADS-box transcription factor-mediated regulation of fumonisin biosynthesis in *Fusarium verticillioides***

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*Fusarium verticillioides* is an important maize pathogen, leading to Fumonisin B1 (FB1) in infested kernels. MADS-box transcription factors (TF) were found to modulate polyketide synthase (PKS) gene expression and FB1 production in *F. verticillioides*. With next-generation sequencing of *F. verticillioides* wild type and MADS-box TF mutant cultures, we used a suite of computational network-based tools, e.g. partial correlation, log-likelihood

ratio matrix and seed-and-extend approach, to perform a system-oriented transcriptome analysis to predict downstream genetic subnetwork modules associated with FB1 production. Our aim was to identify and characterize system-level changes across correlated genes rather than simply focusing on individual gene expression. The resulting subnetwork modules are predicted to contain hub genes, which likely play a crucial regulatory role within functional modules. Two predicted subnetwork modules were analyzed *in silico*, and five putative hub genes were subjected to functional characterization. Deletion of RAS GTPase (*FvRSR1*) and methyltransferase (*FvEFM3*) led to a significant reduction in FB1 levels. A comparative qPCR was performed to investigate the impact of FvRsr1 and FvEfm3 on the expression of neighboring genes in two subnetworks, which did show significant changes. Collectively, these results support our prediction that *FvRSR1* and *FvEFM3* serve as a key hub gene in each subnetwork and regulate FB1 biosynthesis.

#### **Independent amplification of a housekeeping gene and its evolutionary significance in the Dothideomycetes**

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Transposable elements (TEs) are key drivers of genome evolution, but the exact mechanisms of how they modulate gene and genome function are largely unknown. We identified an event where a housekeeping gene (histone H3) was captured and amplified by a hAT DNA transposon in the genome of the banana pathogen *Pseudocercospora fijiensis*. Comparative genome analysis in related Capnodiales revealed a similar event occurred in the corn pathogens *Cercospora zeae-maydis* and *C. zeina*, but was lacking in the soybean pathogens *C. sojae* and *C. flagellaris*. Histone H3 amplification had been described previously in Pleosporales (*Pyrenophora tritici-repentis*). Our analysis showed key differences in the amplification events between the Pleosporales and Capnodiales, namely, 1) extent of histone H3 amplification, 2) difference in H3 nucleotide composition and 3) number of functional H3 genes present. In the Capnodiales, all amplified H3 copies were inactivated by RIP, which suggests the original H3 gene was under strong negative selection pressure. The interplay of TEs and RIP can result in various outcomes for gene and genome evolution. For instance, when TEs act as breeding grounds for pathogenicity genes, such interactions can influence gene function and/or regulation, and ultimately affect host-pathogen interactions.

#### **Transcriptomic analysis identifies specificity in *Fusarium verticillioides* metabolic response to *Bacillus mojavensis* lipopeptides**

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*Fusarium verticillioides* is a mycotoxigenic fungus capable of both pathogenic and asymptomatic endophytic lifestyles in maize; such intimate association renders efficient chemical control cost-prohibitive. *Bacillus mojavensis* RRC101 is a maize endophyte demonstrating both *in vitro* antagonism of *F. verticillioides* and *in planta* reductions of disease and mycotoxin accumulation, the former attributed to production of lipopeptide antibiotics. Although both RRC101 surfactins and fengycins induce increase mycotoxin accumulation in plate assays, only fengycins are antagonistic and induce hyphal distortion, pigment accumulation indicative of a stress response, and violent lysis. Preliminary analysis of RNA sequencing data has identified common functional groups in transcripts enriched in *F. verticillioides* under fengycin antagonism, particularly structural proteins and hydrolytic enzymes. Genes responsible for secondary metabolism, specifically antibiotic production, are also upregulated under lipopeptide challenge. These data suggest that differential lipopeptide responses previously observed in *F. verticillioides* reflect an underlying cross-kingdom “conversation” of secondary metabolites between maize endophytes.

#### **Temporal succession of an *Aspergillus flavus* biocontrol agent in commercial cornfields in Texas**

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*Aspergillus flavus* contaminates corn by producing carcinogenic aflatoxins (AF). This results in devastating economic losses worldwide and poses serious health risks. The most effective control strategy for reducing AF contamination is the application of non-aflatoxigenic strains of *A. flavus*, such as NRRL 21882 (=Afla-Guard®) or NRRL 18543 (=AF36). However, we have a limited understanding of the succession of biocontrol agents in cornfields. From a genomic perspective, we are examining the influence of NRRL 21882 on the populations of *A. flavus* from Texas commercial cornfields. In 2014, *A. flavus* strains were isolated from kernels from fields that were not treated or treated with NRRL 21882 in 2011. To obtain fine resolution of genetic variation, we conducted genome-wide sequencing, which allowed us to detect changes in the genomes of NRRL 21882-derived strains and introgression of NRRL 21882 into native strains over a three year period. From an analysis of 168 *A. flavus* strains, 32 strains are missing their entire AF gene cluster, a marker of NRRL 21882; 14 of these were in untreated fields. Eight of the strains missing the cluster are putative recombinants, phylogenetically grouping with full cluster strains. Ongoing sexual reproduction is indicated by approximately equal distributions of both mating types in treated and untreated fields.

#### **Comparative genomics of S and L morphotypes of *Aspergillus flavus***

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Strains of *Aspergillus flavus* vary greatly in their level of aflatoxin production and atoxigenic strains are not uncommon in nature despite the fungus' notoriety for producing the carcinogenic mycotoxin. *Aspergillus flavus* strains are of two morphotypes; the S morphotype that produces numerous small sclerotia and limited conidiospores and the L morphotype that produces relatively fewer large sclerotia and abundant conidiospores. S strains are consistently toxigenic, whereas L strains vary greatly in toxin production ranging from atoxigenic to highly toxigenic. This suggests the two morphotypes have adapted to different niches where selection pressure to maintain aflatoxin production is high in the environment the S strains inhabit, and low in which the L strains inhabit. To develop hypotheses on differential niche adaptation, we compared the genomes of three S strains and three L strains to identify genes unique to each morphotype. A notable difference in secondary metabolite genes was observed and the results from the comparative genomics analyses will be presented.

#### **Functional characterization of candidate effector proteins identified from the wheat scab fungus *Fusarium graminearum***

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Fungal pathogens often produce certain small secreted cysteine-rich proteins (SSCPs) during pathogenesis that may function in triggering resistance or susceptibility in specific host plants. We have recently identified a total of 190 SSCP genes encoded in the genome of the wheat scab fungus *Fusarium graminearum* and found at least 23 SSCP genes to be true extracellular proteins that are expressed *in planta*. To facilitate functional characterization, we compared the expression patterns between compatible and incompatible interactions by transcriptional analysis. A majority of SSCP genes examined were found to be expressed in identical or similar patterns when the fungus attacked susceptible wheat cultivars such as Grandin and Wheaton. However, the expression patterns of at least 13 SSCP genes were altered when the same pathogen infected the resistant cultivar Sumai 3: the expression of 11 SSCP genes appeared to be suppressed or “down-regulated” at certain stages of infection while two others were apparently “up-regulated” at 4-8 days post inoculation. Gene knock-out experiments are underway with the priority given to these differentially expressed SSCP genes. The resultant deletion mutants will be subjected to pathogenicity assays on both susceptible and resistant wheat cultivars to determine the roles of these candidate effector proteins in *F.*

*graminearum*-wheat interactions. This study may provide new molecular insights into *Fusarium* head scab, a devastating disease of wheat crops worldwide.

#### **Analysis of a ketide synthase from fungal endophytes of toxic locoweeds**

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Toxic *Astragalus* and *Oxytropis* locoweeds are legumes in which toxicity is due to fungal endophytes of *Alternaria* section *Undifilum* sp. (*A. sec. U. sp.*). The fungi produce swainsonine, an alkaloid  $\alpha$ -mannosidase inhibitor that causes locosim disease when consumed by grazing animals. Polyketide synthase (PKSs) is a key enzyme in swainsonine biosynthesis. KS is the most highly conserved and informative of the multi-domain enzymes for predicting pathway associations. The objective of this study was to analyze the KS sequences from *A. sec. U. sp.* isolated from locoweed plants of *Astragalus lentiginosus* var. *diphysus*, *lentiginosus*, *araneosus*, and *wahweapensis*, and *A. mollissimus* var. *biglovii*, *earleii*, *wootoni* and *mollissimus* that were collected from the western USA. The KS from these endophytic fungi were compared to *A. sec. U. oxytropis* isolated from *Oxytropis lambertii* and *O. sericea* using PCR. A 741 bp amplicon was sequenced and analyzed to produce maximum parsimony trees. Fungi isolated from different varieties of locoweed and locations had different morphologies and colors in culture. The KS sequences were highly conserved across *A. sec. U. sp.*, but differed slightly between *A. sec. U. oxytropis* and *A. sec. U. fulvum* and *A. sec. U. cinereum*. Comparing the KS from additional locoweed endophytes and *A. sec. U. sp.* will allow the further examination of phylogenetic relationships among these fungi.

#### **A metabolomic approach to investigate the putative role of drought stress in Esca disease of grapevine**

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Esca is a destructive disease of grapevine known to be associated with several xylem inhabiting fungi, namely *Phaeoaniella chlamydospora* (*Pch*) and *Phaeoacremonium alephilum* (*Pal*). However, the disease mechanism(s) remains unclear. These fungi seem capable of living for several years as endophytes without apparently causing the disease. It is unknown why these fungi become pathogenic, but symptom appearance has been associated with abiotic factors, particularly water stress. We hypothesize that changes induced by drought on the components of xylem sap contribute to symptom appearance.  $^1\text{H}$ -Nuclear Magnetic Resonance spectroscopy coupled with multivariate statistical techniques was used to investigate the interaction of drought stress and Esca-associated pathogen infection. Young, greenhouse-grown, *Vitis vinifera* cv. Chardonnay cuttings were inoculated with *Pch* or *Pal* six weeks before inducing drought stress. Xylem sap was collected thirteen weeks after inoculation. Drought stress induced major changes in several compound classes, e.g. amino acids, carbohydrates and aromatic compounds. The modulation of these metabolic changes by fungal inoculation is being considered. This “omic” approach allowed to gain better insight into the biotic-abiotic interaction, and suggested candidates that could be tested in the control of esca-associated fungi, thus leading *Science* closer to *Practice*.

#### **PiCS, a predicted protein-protein interactome of the frog eye leaf spot pathogen *Cercospora sojina***

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Frog eye leaf spot (FLS) is a foliar disease of soybean caused by the fungus *Cercospora sojina* Hara. FLS is a severe problem for soybean production, particularly in the mid-southern United States causing millions of dollars in losses every year. A predicted protein-protein interaction (PPI) map provides an overall snapshot of all the protein interactions occurring in an organism. Therefore, the development of PiCS, a predicted protein-protein interactome of *C. sojina*, can decipher many of the primary metabolic processes, biological processes and developmental processes in the fungus. This will help further our understanding of the mechanisms governing pathogenesis in the *C. sojina* – soybean pathosystem. PPI maps have been generated for some model organisms such as human, fruit fly, and arabidopsis using high throughput experimental methods. In this study, we used PPI data from 11 model organisms to predict the PPI map in *C. sojina* via the interlog method using the INPARANOID and ENSEMBL algorithms. PiCS is comprised of 4,092 proteins (nodes) and 144,488 interactions (edges). It covers 33% of the total *C. sojina* proteome. Subnetworks were also built for secreted proteins in *C. sojina* as well as for putative pathogenicity related genes. This is a first attempt to build a PPI map for a member of the *Cercospora* genus. It is a new tool that could be used to understand the interaction between soybean and *C. sojina*.

#### **Comparative analysis of predicted proteomes from forma speciales of *Fusarium oxysporum* causing palm wilt**

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*Fusarium oxysporum* f. sp. *palmarum* [FOP] and *Fusarium oxysporum* f. sp. *canariensis* [FOC] cause lethal fusarium wilt of palms in Florida. While FOC and FOP cause similar diseases, FOP has a wider host range and appears to be more aggressive than FOC. The focus of the current study is to compare the predicted FOC and FOP proteomes with various formae speciales of *F. oxysporum* to identify common and unique gene families. To date, the FOP and FOC genomes were sequenced, assembled and annotated using multiple bioinformatic approaches. Genome sizes of FOP and FOC are 46.9 Mbp and 46 Mbp, respectively, and the predicted proteomes consist of 15528 (FOC) and 15974 (FOP) proteins. CAZy analysis indicate FOP and FOC share similar copies of sugar transporters, cellulase, and secretory lipase, whereas FOP has additional copies of necrosis inducing proteins, cysteine rich secretory proteins and cell degrading enzymes, such as cutinase and pectate lyase. OrthoMCL was used to cluster related proteins among *Fusarium* genomes, resulting in 396 (FOC) and 511 (FOP) unique clusters. Secondary metabolite genes were present in 22 OrthoMCL clusters. Two gene clusters related to nonribosomal peptide synthases have increased copy number in FOP, indicating the possible role of toxins in pathogenicity. Further annotation is underway to identify putative functions of unique and common genes and to develop hypotheses concerning differences in pathogenic specificity.

#### **Fungal endophyte community analysis of green coffee beans: A comparison across growing regions and qualities**

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Coffee is considered a global dietary staple due to its distinct flavor, longstanding cultural significance, and stimulating caffeine content. During the production process, defective green coffee beans are sorted out so the batch contains below a certain percentage of them, as a non-homogenous batch can negatively impact the roasting process. A reduction of this percentage of defects would result in higher yields and better quality coffee. We hypothesize that there are notable differences in the endophyte communities between good and defective green coffee beans, and one or more fungal taxa can be associated with bean health or with particular economically significant defects. Through next-generation amplicon sequencing, we analyzed the endophytes present within beans from six defect categories across three coffee growing regions and made comparisons to identify the fungal taxa associated with these categories. We identified a variety of fungal communities present within the samples and were able to make statistical associations between both healthy and defective beans and certain endophytic taxa. Particularly, our studies indicate that multiple species from the *Pichia* genus may be responsible for promoting healthy bean development. Our findings will open the door to new research regarding the manipulation of endophytic fungi to improve overall coffee production and quality.



### **Transcriptomic response of *Fusarium verticillioides* associated with nitrogen availability and development of sugarcane pokahh boeng disease**

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Pokahh boeng, caused by *Fusarium verticillioides*, is a serious disease in sugarcane industry. The impact of nitrogen source (ammonium sulfate, urea or sodium nitrate) on sugarcane pokahh boeng disease and its pathogen was investigated *in planta* and fungal growth and sporulation production was measured *in vitro*. The results showed that ammonium and nitrate were beneficial to fungal mycelium growth, cell densities and sporulation and enhanced the disease symptomology of sugarcane pokahh boeng in comparison to urea fertilization. Total 1,779 transcripts out of 13,999 annotated genes identified from global transcriptomic analysis by RNA sequencing were differentially expressed in *F. verticillioides* CNO-1 grown in the different sources of nitrogen. A subset of these genes were associated with organic nitrogen assimilation and reproductive processes of *F. verticillioides*. The identified DEGs suggested the modes of nitrogen uptake and assimilation for *F. verticillioides* during different nitrogen regimes. Our results further demonstrated that nitrogen availability might play an important role in disease development by increasing fungal cell growth as well as influencing the expression of genes required for successful pathogenesis. These genes included those associated with phenotypic alteration, reproduction, nitrogen and carbon metabolism as well as several transcription factors responsible for these metabolisms.

### **Metagenomic analysis of oomycete communities in rhizosphere soil from field pea on the Canadian prairies using Illumina Miseq**

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Oomycetes include several important soil-borne pathogens that cause damping-off and root rot in many crops including pea. Samples of soil from the rhizosphere of pea plants were collected from patches of healthy or diseased plants (127 samples from 26 commercial fields) on the Canadian prairies in 2013 and 2014. Oomycete communities were characterized using metagenomic analysis of the ITS1 region on Illumina Miseq. Roughly 4 million high quality sequences were obtained. Clustering took place at 85% coverage and 97% similarity. A representative sequence of each operational taxonomic unit (OTU) was compared to NCBI sequences. Species were identified at (minimum) 90% coverage and 97% similarity, and genera at 80% coverage and similarity. Clusters below 80% coverage or identity were labelled as unidentified. From 102 identified OTUs, 43 species and 16 oomycete genera were identified. *Pythium* was the dominant genus and *P. heterothalicum* was the most prevalent species. *Aphanomyces euteiches*, a very important pea root rot pathogen, was detected in 71 sites, but in very low abundance ( $\approx 0.2\%$ ). Multivariate analysis revealed difference in oomycete communities of healthy and diseased sites, and among years and provinces. This study shows that deep amplicon sequencing on Miseq can reveal unknown diversity of oomycete communities in agricultural soil, and that primer optimization for higher detection power of economically important oomycetes such as *A. euteiches* is warranted.

### **Apple replant disease and the -omics: Interaction of apple rootstock metabolome and the soil microbiome**

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Apple replant disease (ARD) negatively impacts tree health and reduces crop yield in new orchard plantings. Use of tolerant rootstock cultivars can diminish the growth limiting effects of ARD; however specific rootstock attributes enabling ARD tolerance are not understood. Systems biology tools were used to contrast root exudate biochemical profiles and corresponding soil microbial ecology between ARD tolerant and susceptible rootstock genotypes. A metabolomic approach utilizing LC-MS/MS QTOF was used to characterize water-soluble root exudate metabolites collected periodically from water percolated through rootstock roots planted in pasteurized quartz sand over 12 weeks. Soil microbial community profiling was conducted by terminal restriction fragment length polymorphism (TRFLP) or next-generation sequencing (NGS) on samples collected concurrently from soil with a history of ARD treated daily with the flow through exudates from rootstocks cultivated in quartz sand. Results indicate rootstock cultivars differ in both root exudate composition and quantity, and correspondingly soil microbial communities are altered in a rootstock genotype-dependent manner. Differences in exudate metabolome associated with ARD cultivar tolerance, and temporal dynamics of root exudate production and microbial populations during early stages of rootstock growth following dormancy, may offer insight into ARD tolerance and the early stages of disease development.

### **Differential metabolome analysis of field-grown maize kernels in response to drought stress**

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Drought stress constrains maize kernel development and can exacerbate aflatoxin contamination. In order to identify drought responsive metabolites and explore pathways involved in kernel responses, a metabolomics analysis was conducted on kernels from a drought tolerant line, Lo964, and a sensitive line, B73, with and without drought stress treatment for 7 and 14 days after drought induction beginning 7 days after pollination. Using ultra-performance liquid chromatography coupled with MS/MS, we profiled 445 metabolites covering 42 pathways. Drought stress induced higher accumulation of glycerolipid and phospholipid metabolites, and decreased content of galactolipid, amines and polyamines, amino sugar, and nucleotide sugar metabolites in both lines. However, B73 was found to accumulate fewer metabolites involved in dipeptide and pyrimidine metabolism under drought stress than Lo964. Lo964 also exhibited increases in sphingolipid, sterol, and purine metabolism in comparison to B73. Collectively, higher sugar accumulation, a lower rate of energy metabolism, and elevated lipid and nucleic acid turnover was observed in B73. Conversely, higher amino acid and dipeptide accumulation, a lower TCA cycle rate, and elevated lipid and nucleic acid turnover was observed in Lo964 in response to drought stress. These results suggest that drought sensitivity is associated with differential metabolite accumulation, and diverse metabolic pathways in maize kernels.

### **Comparison of *Fusarium graminearum* transcription factors affecting *Fusarium graminearum* virus 1 accumulation based on phenome analysis**

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The *Fusarium graminearum* virus 1 (FgV1) causes hypovirulent phenotypic changes in *Fusarium graminearum*. It has been reported that the numerous *F. graminearum* genes were differentially expressed upon FgV1 infection including genes encoding regulatory factors. Using gene-deletion mutant library of 657 putative transcription factors (TFs) in *F. graminearum*, we transferred FgV1 by hyphal anastomosis to screen transcription factors that might be associated with viral replication or symptom induction. FgV1-infected TF mutants were divided into three groups according to the mycelial growth phenotype compare to the wild-type FgV1-infected strain (WT-VI). The FgV1-infected TF mutants in Group 1 exhibited slow or weak mycelial growth compare to that of WT-VI on CM media at 5-dpi. In contrast, Group 3 consists of virus-infected TF mutants showing faster mycelial growth and mild symptom compared to that of WT-VI. The hyphal growth of FgV1-infected TF mutants in Group 2 was not significantly different from that of WT-VI. These growth differences among the FgV1-infected TF mutant groups might be related with FgV1 RNA accumulations in infected host fungi. To confirm this, we tested FgV1 RNA accumulation of some of FgV1-infected TF mutants in each group. Taken together, our analysis will help us identifying regulator(s) of FgV1-triggered signaling and antiviral responses and thus expand our understanding on complex regulatory networks between FgV1 and *F. graminearum* interaction.

### Root transcriptome analysis reveals viral diversity in two species of blueberry

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A growing number of metagenomics based approaches have been used for the elucidation of viral diversity in insects, cultivated plants, and water in agricultural production systems. However, while the generation of plant transcriptomes has greatly increased over the last few years, their use for data mining viral sequences has been largely unexplored. In this study, blueberry root transcriptomes (8 libraries each of *Vaccinium arboreum* and *V. corymbosum* x *V. darrowi* 'Emerald') were analyzed using bioinformatic analyses for plant viral sequences. *De novo* assembly of 'Emerald' libraries yielded a complete viral genome of the *Blueberry red ringspot virus* (BRRV), genus *Soymovirus*, family *Caulimoviridae*, a plant virus with a dsDNA genome. BRRV was detected by PCR in 100% and 25% of the 'Emerald' and *V. arboreum* samples, respectively. Since sequences of species in the *Caulimoviridae* are known to be present as integrants in their hosts, back to back primers were designed to detect the episomal form of BRRV genome. The episomal form of BRRV was detected in root tissue of 'Emerald' but not *V. arboreum*, which indicated that the BRRV sequences in *V. arboreum* are most likely those of integrants. In addition, BLASTx analysis of 16 blueberry libraries revealed contigs that match sequences in 20 virus genera and 10 families. Overall, this study demonstrates the feasibility of using available host transcriptome data for mining viral sequences.

### Replication and transcription are independently modulated for each *Banana bunchy top virus* DNA component

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*Banana bunchy top virus* (BBTV) causes a severe disease in banana world-wide. BBTV is a single-stranded DNA virus with a genome consisting of six circular molecules of ~1kb each. Some BBTV isolates also carry satellite DNA molecules that are not essential for BBTV infection. To understand the coordinated actions of the multiple genomic components during BBTV infection, we measured the relative and absolute amounts of each DNA component of isolate B2 and its transcript levels by real-time PCR (qPCR) and reverse transcription (RT)-qPCR, respectively. Significant differences in the absolute amounts and expression levels were found among BBTV genomic components. The most abundant DNA molecules are those of DNA-U3, DNA-M, and DNA-N, 4 to 6 times more than those of DNA-R. The least abundant DNA molecules are those of DNA-C, 12 times less than that of DNA-U3. Interestingly, two satellite DNA components are also present at high levels, with amounts similar to that of DNA-U3. Transcript levels determined by RT-qPCR did not correspond to the absolute amount of each DNA component. The most abundant transcripts came from DNA-N, followed by DNA-U3, and DNA-S, and the least abundant transcripts came from DNA-C, 110-fold less than those from DNA-N. The ratios of transcript copies over DNA copies ranged from 0.15 to 2.63. These data suggest that both replication and expression levels are independently modulated for each DNA component, possibly by cis-acting DNA sequence elements.

### Identification of defense-related genes associated with tomato Sw-7 line against Tomato spotted wilt virus in tomato through transcriptome analysis

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Tomato spotted wilt virus (TSWV) is a highly infectious tospovirus, and one of the most damaging plant viruses infecting tomatoes worldwide. Developing a tomato cultivar with TSWV-resistance would be the most effective approach for disease management. Comparative analysis of differential expression of genes in plant functional pathways between highly isogenic tomato breeding lines with striking difference in resistance and susceptibility to TSWV may lead to understanding of the mechanism of disease resistance. In the present study, RNA-seq was performed on samples from a near-isogenic breeding line containing *Sw-7* and its recurrent TSWV-susceptible parent at 0, 4, 7, 14, 21 and 35 days post inoculation (dpi). Comparative transcriptome analysis revealed a large number of genes that are differentially expressed (DE) by TSWV infection over time, including pathogenesis related 1 (PR1) and 5 (PR5) (Osmotic-like protein), Pto-like, Serine/threonine kinase protein, N-methyltransferase-like protein, auxin, glycine/proline rich protein, MADS box transcription factors, histidine kinase, histone proteins, as well as the RNA silencing pathway genes, argonaute, DCL3 and PolIV and microRNA target genes. Genome-wide transcriptome analysis offers us an opportunity to understand the mechanism of disease resistance, which may lead us to an effective management of TSWV in tomato and other crops.

### Transcriptome profiling to discover defense-related genes associated with resistance line ty-5 against Tomato yellow leaf curl virus in tomato

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Tomato yellow leaf curl virus (TYLCV), a whitefly-transmitted begomovirus, has caused serious economic losses to tomato crops in the U.S. and around the world. The most effective management would be the use of a TYLCV-resistant tomato cultivar. Several sources of TYLCV resistance genes have been identified and incorporated in tomato breeding. The *ty-5* gene recently discovered in 'Tyking' was a recessive gene. Although its function has not been well-characterized, near-isogenic breeding materials with TYLCV resistance have been generated. In the present study, after feeding by viruliferous whiteflies (*Bemisia tabaci*, MEAM1) at 0, 4, 7, 14 and 21 days post inoculation (dpi), leaf materials were collected on the test tomato plants for RNA-seq. Differentially expressed (DE) genes based on genome wide transcriptome profiling were analyzed and categorized based on their functions. Those defense-related genes included pathogenesis related 1 (PR1) and 4b (PR4b), CC-NBS-LRR, defensin protein, and heat shock protein were differentially expressed in the *ty-5* line. Furthermore, RNA silencing and transcriptional silencing pathway genes, such as RdRp family protein, DNA (Cytosine-5)-methyltransferase 3 and microRNA target genes were also identified. To conclude, the genome wide transcriptome analysis allows us to have a global view on the differential gene expression, which may lead us to an effective management of TYLCV through breeding for durable disease resistance cultivars.

### National Seed Health System: Standardizing seed health practices in the United States for seed exports

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The U.S. National Seed Health System (NSHS) is a program authorized by USDA-APHIS and administered by the Iowa State University Seed Science Center in Ames, Iowa. NSHS provides accreditation to non-government entities to perform certain activities needed to support phytosanitary certification for the international movement of seed, and conducts peer review of protocols for approval as standard phytosanitary seed health testing methods. NSHS is governed by a Policy and Procedures Advisory Board in collaboration with the USDA-APHIS Seed Health Accreditation Manager and the National Plant Board Council/PPQ Strategy Team. Currently, there are 19 entities accredited by NSHS to perform field inspections, seed sampling and/or the seed health testing necessary to obtain phytosanitary certificates for exporting seeds. These organizations include private seed companies, state crop improvement associations and private testing labs. NSHS has been operating since 2001 and has approved 86 methods as standards for testing seeds for 66 different pathogens within a range of vegetable and field crops. NSHS works closely with international organizations to harmonize seed health testing methods that are accepted around the globe for phytosanitary certification. The standardization of seed health methods and practices increases confidence in the health status of US seed exports and facilitates safe and efficient international movement of seed for research and commercial purposes.

### **Implementation of a Quality Management System in an ISO 17025 Accredited Plant Pathogen Diagnostic Laboratory**

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In 2015, the USDA-APHIS-PPQ-S&T Beltsville lab (Bldg 580) became the first ISO 17025:2005 accredited regulatory plant pathogen diagnostic lab in the United States. The ISO/IEC 17025 Standard is used world-wide by testing labs in the human clinical realm to the Federal Bureau of Investigation criminal analysis labs to ensure accuracy and reliability of test results. As a result of incorporating a system for technical and quality management, the diagnostics and results generated by the lab are internationally recognized to be technically competent, traceable, repeatable and statistically valid. The implementation of a Quality Management System (QMS) required the establishment of a systematic approach to control all laboratory processes as well as well-defined work instructions that were developed using statistically sound validation and verification data. The newly implemented Laboratory Information Management System (LIMS) has been integrated into the Laboratory QMS to provide complete sample, equipment, reagent and report tracking. This provides an added level of confidence that all data is sufficiently documented, reproducible, and traceable. An annual assessment by an accreditation body ensures that our laboratory continues to meet the requirements of technical competence. Continual improvements to the lab and its commitment to stakeholders through use of the QMS are now an operational component of the USDA-APHIS-PPQ-S&T Beltsville Lab.

### **eFile and the USDA Animal Plant Health Inspection Service's (APHIS) PPQ Pest Permitting Process**

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USDA APHIS safeguards U.S. agriculture against the entry, establishment, and spread of economically and environmentally significant pests. APHIS PPQ uses a permit type called the "PPQ 526 permit" to regulate the interstate movement and importation of plant pathogens (such as bacteria, fungi), pests (such as insects and nematodes), and biocontrols. We currently issue permits through ePermits, a web-based system that allows users to submit and track applications. PPQ receives over 6,000 of these applications per year. The average processing time is 80 days. Depending on the risk of the organism(s) requested, we may require that the work to be done in a USDA APHIS- inspected containment facility to adequately contain the organisms. This may increase processing time as PPQ determines if the facility is adequate. In most cases, this applies for the receipt of foreign organisms, federal quarantine pests, and diagnostic facilities. APHIS is developing a new permitting system known as "eFile" which we will deploy soon. We expect that this new system could substantially reduce the processing time for certain permits.

### **National Clean Plant Network for Roses**

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At the urging of the grape and fruit tree industries, the National Clean Plant Network (NCPN) was established to provide high quality asexually propagated plant material free of targeted plant pathogens and pests for growers in the United States. In 2008, the NCPN was initiated with grapes and fruit tree crops. This was followed by the addition of citrus, berries and hops in 2010 and roses and sweet potatoes in 2015. Each specialty crop has its own commodity network composed of representatives from industry, academia, extension, state government and other interested experts. This program supports translational research, education and extension initiatives to maintain the network's high quality collections and strengthen its services. Since being established in 2015, the National Clean Plant Network for Roses (NCPNR) has been working to augment, renovate and improve the current rose collection at the Foundation Plant Services (Davis, CA), to assess the frequency of viruses in roses in the USA, to develop educational materials, and to establish the best protocols for determining the pathogen status of a rose.

### **Master in Plant Health Management: Education and training to meet 21st century trends and challenges in Extension and industry**

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In response to the growing demand for qualified professionals to address emerging trends and challenges in plant health, the Master in Plant Health Management (MPHM) was established by the Department of Plant Pathology and the Department of Entomology at The Ohio State University. The MPH program, which is affiliated with the Professional Science Master's Association, is distinguished by an interdisciplinary curriculum that integrates agricultural science coursework with professional development and workforce skills in the College of Food, Agricultural, and Environmental Sciences. An online option was added in 2014. The program has attracted a diverse population of students, many of whom are working professionals and non-traditional students. A new graduate associateship program was established with OSU Extension to recruit, educate and support future Extension educators to work in the field of agriculture and natural resources. Students work directly with Extension teams and mentors on projects that include agronomic field trials, ornamental pathology, and citizen science programs. The MPH program's placement rate is very strong, with graduates working in Extension, industry and research.

### **Virtual disease management using Blightpro DSS**

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Experiential learning activities can support student understanding of material in an introductory plant pathology course where student base knowledge related to content can be limited. Unfortunately not all course content can be successfully translated into authentic hands-on activities. Computer-based activities such as simulations can be successful in meeting this challenge. We developed a laboratory exercise using a practice version of BlightPro DSS (Decision Support System), which models the progress and control of potato late blight epidemics. The major learning outcome for students is to synthesize and apply their knowledge of disease management to the "real-time" management of late blight in relation to weather, cultivar resistance, and fungicide application. Working in small groups, students developed fungicide schedules to protect their potato crop from late blight while minimizing economic costs and environmental impact. A class discussion on the outcome of student group data concluded the exercise. Students demonstrated a strong cognitive presence throughout the exercise, thinking critically about plant disease management. They sought information from instructors and electronic resources during the exercise, beyond what was required for completion. Student collaboration in small groups promoted cooperative learning opportunities with students sharing previous experiences and knowledge applicable to the exercise outside of content presented in lecture.

### **Seed treatment with multiple rhizobacterial strains promotes soybean growth and suppresses reproduction of soybean cyst nematode in the field**

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The soybean cyst nematode (*Heterodera glycines*, SCN) is the most damaging pathogen of soybean (*Glycine max*) worldwide. Seed coating with nematicides or biological control agents represent a valuable method for controlling nematode pests. Here, we presented the study that evaluated the effects of combining three rhizobacterial strains, including *Sinorhizobium fredii*, *Bacillus megaterium*, and *Bacillus simplex*, as a seed treatment on SCN control and soybean yield enhancement under greenhouse and field conditions in northeast China. An optimal formulation consisting of different proportion of the above three rhizobacterial strains was determined based on the results of the germination vigor of the coated soybean seeds and subsequently designated as SN101. SN101 coated soybean seeds were then used in greenhouse studies and field trials conducted in two field locations for a period of three years. After each growing season, SCN reproduction was determined by nematode egg and cyst numbers and soybean growth and yield were also evaluated. Our results showed that SN101 treatment greatly inhibited SCN reproduction and significantly promoted plant growth and yield production in both greenhouse and field trials, suggesting that SN101 is a promising seed-coating agent that may be used as an alternative tool for controlling SCN in soybean fields.

#### **Rapid differentiation of *Claviceps* species occurring in Oregon and Washington using high resolution melting curve analysis**

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Ergot disease of grass seed crops can be caused by *Claviceps purpurea* and *C. humidiphila*, a species recently reported to occur in certain regions of OR and WA. Routine identification of *Claviceps* species involves conventional PCR amplification and sequencing of ITS,  $\beta$ -tubulin or EF-1 $\alpha$  genes. In this study, a high resolution melting (HRM) assay was developed to rapidly identify and differentiate *C. purpurea* and *C. humidiphila* based on the melting temperatures (T<sub>m</sub>) of partial  $\beta$ -tubulin amplicons. PCR primers were designed to target and amplify a 110 bp region of the  $\beta$ -tubulin gene containing multiple single nucleotide polymorphisms generating amplicons with distinct melting profiles for each species. Forty-six fungal cultures, previously classified based on RAPD profiles, and ITS,  $\beta$ -tubulin, and EF-1 $\alpha$  gene sequences, were used to validate the HRM assay. The T<sub>m</sub> for *C. purpurea* and *C. humidiphila* ranged between 79.8 to 80.3 and 78.9 to 79.3, respectively. Classification of *Claviceps* species based on T<sub>m</sub> using linear contrasts (P < 0.05) correctly grouped 96% (N=46) of samples previously classified using sequence data. In two cases, *C. purpurea* was misclassified as a mixture of the two *Claviceps* species. Therefore HRM analysis allows rapid detection and differentiation of these two *Claviceps* species and will be useful to study their distributions within OR and WA cool-season grass seed growing regions.

#### **Effect of root pathogens *Bipolaris sorokiniana* and *Fusarium graminearum* on germination and seedling blight in wheat in South Dakota**

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*Bipolaris sorokiniana* (Bs) and *Fusarium graminearum* (Fg) are important wheat root pathogens that effect seed germination and seedlings establishment thus ultimately impact the crop productivity. The objective of this study was to explore the effect of Bs and Fg infested seed on germination and seedlings establishment (blight) of 7 wheat cultivars, Advance, Briggs, Forefront, Oxen, Russ, Prevail, and SD4215 which were planted at Volga, SD in 2015. The treatments included, uninfested + untreated, uninfested + treated with fungicide, infested (Bs) + treated, infested (Bs) + untreated, infested (Fg) + treated, infested (Fg) + untreated. Seed germination and seedling blight data was recorded after the germination for 3 consecutive weeks. Wheat cultivars varied in seed germination and seedling blight to both pathogens; low seed germination (ranging from 35-71%) and high seedling blight (30-65%) was observed in Fg infested seed, whereas, in Bs infested seed the germination ranged 58-100% and seedling blight ranged 0-42%. The cultivars Oxen and Prevail had the lowest (33%) and the highest (71%) seed germination respectively. Also the treatments including the fungicide significantly increased the germination (ranging from 5-44%) and the seedling blight got reduced in almost all the cultivars.

#### **Frequent isolation of *Colletotrichum* species complex from citrus tissues infected with leaf and fruit spot disease in Ethiopia**

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Citrus are economically important fruit crops in Ethiopia with estimated acreage of 7,040 hectares and annual production of 72,459 tons. The citrus leaf and fruit spot is among the major diseases contributing to a low productivity (10.3 t/ha) of citrus in Ethiopia. Therefore, a study was conducted to study diversity and relationships of the pathogens associated with leaf and fruit spot disease in major citrus growing regions of the country. Citrus leaves and fruits samples with distinct leaf and fruit spot disease symptoms were collected from 16 districts of Ethiopia and a total of 167 fungal isolates were recovered and monosporic or hyphal tip cultures were developed for each isolates. The genomic DNAs of all 167 isolates were amplified and sequenced for internal transcribed spacers (ITS), long subunit (LSU) and actin (ACT) genes. The sequences obtained were compared with the GenBank database sequences. The multilocus sequences based phylogenetic analysis revealed that 167 isolates were from either *C. gloeosporioides* or *C. boninense* species complexes. Surprisingly, this study did not recover any *Pseudocercospora angolensis* isolate, the causal agent reported for citrus leaf and fruit spot diseases. The detached leaf assay revealed the ability of both *C. gloeosporioides* and *C. boninense* isolates to cause foliar symptoms on multiple citrus species.