

Experimental strategies to unravel the MDR mechanism in new emergent phenotypes of M. graminicola highly resistant to several DMIs

Selim Omrane, Anne Sophie A. S. Walker, Johann J. Confais, Daniel Martinho, Catherine Lanen, G.H.J. Kema, D. Dedieu, S. Fillinger

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BOOK OF ABTRACTS

8th International Symposium on Mycosphaerella and Stagonospora Diseases of Cereals

Etienne Duveiller and Pawan K. Singh, Editors

September 11-14, 2011 Mexico City, MEXICO







CIMMYT
International Maize and Wheat Improvement Center

8th International Symposium on Mycosphaerella and Stagonospora Diseases of Cereals

BOOK OF ABSTRACTS

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Keynote Address

Sustainable management of *Mycosphaerella graminicola*: approaches and challenges for the French wheat growing industry.

J. Mathieu[1]

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French wheat producers are faced with the double challenge of increasing productivity to contribute to meeting increasing global demand, all the while answering to the French pesticide reduction plan "Ecophyto 2018" which sets an objective of reducing the use of chemical inputs by "50% if possible" by 2018. Management of Septoria tritici blotch (STB), the most important wheat disease in the country, is of prime importance in such a context. After briefly presenting the institutions (Arvalis – Institut du végétal and the Fonds de Soutien à l'Obtention végétale, FSOV) created by French wheat growers to respond to the technical challenges they are faced with, I will illustrate the strategies developed for the industry by Arvalis and its partners to deal with STB. Faced with the difficulties caused by STB resistance to fungicides, Arvalis has organized a network, "Réseau Performances" bringing together extension services and research, comprising approximately 100 field trials per year. This has allowed the combined, continuous monitoring of STB evolution, with regards to QoI resistance and then emergent phenotypes for triazole resistance, and of product performance, as well as disseminating fungicide strategies to slow resistance progress. The institute has also developed decision support tools to aid growers and advisors in safely reducing fungicide use. More recently, Arvalis and the FSOV have invested strongly in evaluating and developing cultivar resistance to STB. These projects will be quickly presented as they are presented in more detail by Gouache et al. in Session 7. Finally, some of the future challenges for improving the management of STB will be discussed, including stimulation of plant defenses.

Oral Presentations

Session 1

Pathogen Biology, Taxonomy, and Evolution

The cytoskeleton in plant infection

G. Steinberg

Department of Biosciences, University of Exeter, UK

Fungi invade plant tissue by polar hyphal growth. This process involves the cytoskeleton, along which vesicles and other growth supplies are delivered to the expanding cell tip. Intracellular transport towards the growth region involves molecular motors, which are nano-machines that utilize ATP to walk along the fibres of the cytoskeleton. Filamentous fungi contain a unique motor protein, myosin XVII, thought to transport secretory chitin-synthase containing secretory vesicles. This myosin motor is also a class V chitin synthase and is a pathogenicity factor in many plant pathogens, including the corn pathogen *Ustilago maydis*. I will summarised the most recent outcome of our work on myosin XVII in *U. maydis* and which explain the cellular importance of myosin VII in pathogenic fungi. I a second part of the talk I will present first results on the morphogenic variability of various strains of *Mycsphaerella graminicola*.

Evolution of Host Specific Toxins in the fungal wheat pathogen *Phaeosphaeria nodorum*

M.C. McDonald[1], P.C. Brunner[1], T.L. Friesen[2, 3], Z. Liu[3], R.P. Oliver[4], B.A. McDonald[1]

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The genetic relationships among *Phaeosphaeria* species that infect wheat are not well understood. This study expanded on earlier work using sequence data to define the species infecting wheat in a global sample. We sequenced 1,835 bp across 3 loci in 309 isolates from around the world, including many isolates from Iran, near the host center of origin,. We were able to differentiate *P. nodorum*, two previously defined taxonomic groups of *Phaeosphaeria avenaria tritici* (Pat), called Pat1 and Pat3 and two additional previously undefined groups, tentatively named P1 and P2. P1 and P2 were found only in Iran. We found some evidence of incomplete lineage sorting between *P. nodorum* and Pat1. We propose these 5 groups as separate but closely related species that are capable of infecting domesticated wheat, but only *P. nodorum* and Pat1 were widely distributed. The main virulence mechanisms identified in *P. nodorum* are host selective toxins (HSTs), which interact in a gene-for-gene manner with toxin sensitivity genes in wheat to cause lesion formation. We found that global populations of wheat-infecting *P. nodorum* carried *SnTox3*, *SnToxA* and *SnTox1* with widely varying frequencies. In a global sample of over 1000 isolates the multi-toxin genotypes did not differ significantly from frequencies expected under random mating. Furthermore, the distribution of toxin sequence diversity did not coincide with the distribution observed for neutral markers in *P. nodorum.* By combining the species phylogeny with data on toxin distribution, we could elucidate the evolutionary timescales over which host selective toxins evolved to become major contributors to this disease complex in the wheat agro-ecosystem.

Evolutionary history of the mitochondrial genome in *Mycosphaerella* populations infecting bread wheat, durum wheat and wild grasses

S.F.F. Torriani, P.C. Brunner, B.A. McDonald

ETH Zürich, Institute of Integrative Biology, Plant Pathology, LFW B25, 8092 Zürich, Switzerland

Improved sequencing technologies have greatly enhanced our ability to understand the genomic evolution of plant pathogens. The recently released genome of *Mycosphaerella graminicola* and subsequent genomic comparison with the closest known progenitor species, named *M. graminicola* S1, are just two of a growing multitude of comparisons. Here we present the unique features of the mitochondrial genome of *M. graminicola.* Two isolates (IPO323 and STBB1) have been completely sequenced, compared and annotated and with addition of 35 partially re-sequenced isolates, revealing one of the lowest levels of intraspecific mtDNA diversity ever published. Another unique feature in the mtDNA of *M. graminocola,* in comparison to other members of the fungal kingdom, is the complete absence of introns. This is remarkable, especially if compared to the largely invaded mtDNAs of *Podospora anserina* or *Leptosphaeria maculans.* Previous analyses based on sequence variation at six nuclear loci indicated that *M. graminicola* diverged from an ancestral population adapted to wild grasses during the process of wheat domestication approximately 10,500 years ago. We tested this hypothesis by conducting coalescence analyses based on four mitochondrial loci, using 143 isolates, including four closely related pathogen species, originating from four continents. Pathogen isolates from bread and durum wheat were also included to evaluate a putative emergence of specificity towards these hosts in *M. graminicola*. Although mitochondrial and nuclear genomes differed greatly in degree of genetic variability, their coalescence was remarkably congruent, supporting the previously reported phylogeny based on nuclear markers. The coalescence analysis was unable to trace *M. graminicola* host specificity through recent evolutionary time, indicating that the specificity towards durum or bread wheat has a recent origin.

Oral Presentations

Session 2

Epidemiology and Population Biology

Population processes of Septoria and Stagonospora diseases of wheat on multiple time-scales

M. Shaw

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Observations by Fraaije and co-workers from long-term wheat experiments at Rothamsted in England suggest that the balance between *Mycosphaerella graminicola* and *Phaeosphaeria nodorum* has swung between the organisms on century time-scales, and that pathogenic characteristics have jumped between alternative states. Likely drivers of these changes include both pollution and varietal changes. To clarify how these would have affected the data and test hypotheses we need models. For short-term forecasting purposes, it is common to limit pathogen population linearly by a fixed or exogenous host area and then link the rate of multiplication of the pathogen to weather. This is manifestly wrong on scales longer than months. Over longer time-scales, we can begin with a spatially uniform host and pathogen and modify standard SIR models. In continuous time this will give logistic dynamics leading to an equilibrium severity. However, the pathogens have important dynamical features on time-scales less than a year. To capture these, more elaborate models incorporating host dynamics are needed. The result is still a static equilibrium. However, with this degree of elaboration we have models in which we can ask whether variation in external drivers (environmental, host disposition and fixed varietal resistance), rather than internal dynamics, could in principle explain what we see in nature. Environmental effects (pollutants, weather, CO_2, variety etc) enter as variations in parameters controlling the multiplication of the pathogen and/or host. A key feature missing from such models is long-term persistence. If host area drops, or varietal resistance rises, or the environment becomes unfavourable, the pathogen either persists at fairly high levels or disappears altogether. Yet like all ecologically obligate pathogens, *M. graminicola* and *P. nodorum* persist (though evolving) indefinitely. So density-dependence and competition must be included. Data on these is very limited. Most studies of pathogen-host interactions use dense inocula, but critical phases of the life-cycle may involve a few spores per plant. For heterothallic pathogens, the probability of mating has inverse density-dependence; this raises another way in which systems with multiple stable equilibria between host and pathogen may arise: this is consistent with historical changes and observed evolution.

Occurrence of leaf blotch pathogens on wheat in the Nordic countries and potential role of toxin-sensitivity loci in determining susceptibility under field conditions

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Insufficient resistance to leaf blotch diseases is a major yield-limiting factor and the main cause of fungicide application in the Nordic wheat production. The leaf blotch disease complex consists of Stagonospora nodorum blotch (SNB) caused by *Phaeosphaeria nodorum*, tan spot (TS) caused by *Pyrenophora tritici-repentis* and Septoria tritici blotch (STB) caused by *Mycosphaerella graminicola*. Historical records show that SNB dominated as the main leaf blotch disease until the beginning of the 1980s. Since then, STB and TS have taken over as the most important diseases in Sweden and Denmark where mostly winter wheat is grown. TS became common in Central Sweden in the mid 1980's, and in Denmark since 2000, promoted by minimum tillage and wheat-intensive crop rotations. SNB is still important in Finland and Norway where most of the wheat acreage is sown to spring wheat. The first TS epidemics in Finland occurred in 2004, and SNB and TS are now equally common on spring wheat while STB mainly infects winter wheat. SNB is still dominating in Norway, but STB occurs frequently in winter wheat and TS has become important in recent years. In 2009-2010, leaf samples were collected from 111 Nordic spring and winter wheat fields (84 Finnish, 11 Norwegian, 11 Latvian, 3 Swedish, 1 Lithuanian and 1 Estonian field). For 10 sites per field, 10 leaves were collected from 10 randomly selected plants; 100 leaves per field in total. The leaves were dried immediately after collection, and pathogens later identified using a specific PCR test. *P. nodorum* was present in 98%, *P. tritici-repentis* in 88%, and *M. graminicola* in 13% of the investigated fields. Only two of the studied 111 wheat fields had none of the tested pathogens (one from Finland and one from Estonia). A diverse set of about 100 spring wheat and 50 winter wheat cultivars and breeding lines were genotyped with closely linked molecular markers to the toxin sensitivity loci *Tsn1, Snn2, Snn3* and *Snn4* and tested for leaf blotch severity in the field based on natural infection at different field locations in Norway. Preliminary results from the 2010 field data indicate that some of the variation in leaf blotch severity in both spring and winter wheat can be explained by allelic variation at *Tsn1* and *Snn2*. The effects of these loci will be further studied during the 2011 field season in near-isogenic lines from wheat breeding populations, and "fresh-from-the field" results from this testing will be presented at the conference.

Assessment of aggressiveness components of *Mycosphaerella graminicola* on adult wheat plants

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Complete resistance in Septoria leaf blotch (isolate-specific interaction) is detected on seedlings either with detached leaves or whole plants. Assessment of aggressiveness components of *Mycosphaerella graminicola* in controlled conditions close to those observed in field during the epidemic period, is, however, required to estimate quantitative resistance. The objective of this study is the design and validation of a standardized method to assess the development of lesions during a complete infection cycle. To this end, localized infection was obtained on adult plants using moderate inoculum concentrations. Chlorotic, necrotic, sporulating areas and number of pycnidia were then assessed every 3-4 days in greenhouse conditions. The resulting curves were fitted to a Gompertz model. Among the several epidemiological variables extracted from the fitting, those with straightforward biological significance were considered as the most relevant components of aggressiveness: incubation period (time from inoculation to the appearance of first chlorosis), latent period (time from inoculation to first pycnidium), development rate of necrotic and sporulating areas, maximal sporulating area, area under the sporulating area progress curve, pycnidial surface density, and pycnidial sporulation capacity (number of pycnidiospores liberated by a single pycnidia). The method was validated in a greenhouse experiment replicated once (2008 and 2009) by inoculating four popular French wheat cultivars with four *M. graminicola* isolates. Correlations between aggressiveness components, specifically those related to symptom dynamics and sporulation capacity, were calculated. This method will be used in further studies:

1) Assessment of partial resistance via the quantification of isolate × cultivar interactions.
2) Comparison of leaf temperature (really perceived by the fungus) and air temperature as indicators of the length of the incubation period.
3) Identification of trade-offs between aggressiveness components related to symptom dynamic (monocyclic phase) and sporulation capacity (dispersal).
4) Investigation of physiological, temperature-related trade-offs between selected aggressiveness components in winter and summer *M. graminicola* isolates sampled within a local population.

Assessing the leaf blotch disease complex in wheat: A challenge for loss estimation and evaluation of warning systems

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Three primary causal agents are involved in the leaf blotch disease (LBD) complex of Norwegian winter and spring wheat: *Phaesophaeria nodorum*, *Mycospaerella grammimicola*, and *Pyrenophora tritici-repentis*. The dynamics of symptom development, similarity of symptoms caused by each agent, and confounding of disease symptoms by leaf senescence interfere with accurate assessment of disease. Empirical and regression models for disease and yield loss forecasting are only as good as the data upon which they are based. Accurately describing the relationship between symptoms and yield loss is therefore critical to meaningful economic thresholds for management decisions and advisory systems. A general guideline for yield loss and disease severity has been described as 1% yield loss per 1% disease severity on the flag leaf at BBCH stage 70-75. However, several years of field trials in Norway indicate that disease severity can increase exponentially during these developmental stages, making disease severity highly dependent upon time of assessment. LBD severity on flag leaves of the spring wheat variety 'Bjarne' at two different locations in 2010 varied during the above BBCH stages from 27% to 44% and from 4.45% to 23.2%. Different varieties may compensate differently for loss of photosynthetic area on the flag leaf due to leaf blotch pathogens, rendering the general guide line for yield loss inaccurate. Preliminary studies in Norway indicated that the relation between yield reduction (TKW) and disease severity of the flag leaf differed substantially for five different spring varieties and ranged from 0.03 to 1.4 at BBCH 70 and from 0.8 to 4.1 at BBCH 75, at one field site at Aas, Norway in 2010. The causes of the observed variation in the relationship between flag leaf severity and yield reduction are poorly understood. Effects of other diseases are not accounted for by leaf blotch assessments, nor are fungicides applied to reference plots necessarily eliminating all disease effects on yield. Timing of assessments may be as critical as the accuracy of the assessments; making it necessary to time the assessments properly, and distinguish clearly between leaf senescence and leaf blotch symptoms.

Operativeness of the wheat pathogens teleomorphs from its stubble in South America

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Wheat fields in South America are annually affected by several foliar necrotrophic pathogens. Most of the spotting leaf complex diseases are originated and associated in a direct proportional relation to the amount of available stubble from previous crops. Soil conservation practices advise farmers to maintain the stubble above the soil or incorporate it after been fragmented. Burning wheat straw and their residues after harvesting, still is been practiced in some areas, and should disappear in the near future by farmer decision or impose by governments policies toward reduction of global warming, CO2 release, and/or soil conservation issues. In the last years, farmers have more commonly incorporated in their farming systems high yield varieties [5 – 12 t/ha] that also produce high amount or residues [6 to 14 t/ha] and most of it remain as unused stubble. The importance of the teleomorph stages observed on wheat stubble are: genetic recombination– new virulence eventually arise; dissemination- windborne ascospores are more efficient infective propagules than their anamorphic stages and pathogen survival – since pseudo/or perithecia could be seen as a resistance structure that release ascospores differentially throughout the crop season. The identified pathosystems associated to the stubble in South America are: *Mycosphaerella graminicola/Septoria tritici* (Septoria Leaf Blotch), *Phaeosphaeria nodorum/Stagonospora nodorum* (Stagonospora nodorum blotch), *Giberella zeae/Fusarium graminearum* (Fusarium head Blight), *Lewia infectoria/Alternaria infectoria* (Black Point and Minor Leaf Spot), *Pyrenophora tritici repentis/Drechslera tritici repentis (*Tan Spot), *Mycosphaerella tassiana/Cladosporium herbarum (*Minor Leaf spot), *Pleospora herbarum/Stemphyllium botryosum* (Minor Leaf Spot), Tapesia *yallundae/Pseudocercosporella herpotrichoides* (Sharp Eye Spot), *Cochliobolus sativus/Bipolaris sorokiniana* (Bipolaris Leaf Spot) and the recently reported and major tread to the Americas *Magnaporthe grisea/Pyricularia grisea* (Pyricularia Blast). A survey of the operativeness of the wheat pathogens teleomorph in South America is presented and discussed.

Is leaf temperature the actual thermal driver of *Mycosphaerella graminicola* development?

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In foliar fungal diseases, leaf temperature is the actual temperature perceived by the pathogen during its development. Due to the leaf energy budget, leaf temperature may differ greatly from the air temperature. The difference depends on the canopy architecture, but also on the climate features (cloudiness, wind regime, etc). Such differences can generate prediction errors in epidemics models, whose development have been over the past 30 years based on the air temperature recorded from weather stations. We hypothesize that the reliability of models could be improved by taking into account the leaf temperature. To test this assumption on *Mycosphaerella graminicola*, we first established the response of STB development at the leaf scale to both wheat leaf and air temperature; then we will study the impact of non-linear responses on STB models. A two-year experiment was carried out in growth chamber (stationary microclimate regime) and greenhouse (fluctuating regime) to characterize the response of STB to temperature on adult plants. Three *M. graminicola* isolates were used to inoculate 576 leaves. The plants were placed for 72 hr in conditions conducive to infection. Every 2-4 days, chlorotic, necrotic, and sporulating areas were estimated on each inoculated leaf, allowing the calculation of incubation and latent periods. Infrared heat lamps positioned above the leaves enhanced the range of mean leaf temperature (10-23°C) without modifying light incidence and the regulated air temperature. The temperature of each leaf was measured with T-type thermocouples during the whole experiment. These experiments have enabled the assessment of the dynamics of STB symptoms for a wide range of leaf temperature. First results have shown that *M. graminicola* is clearly more sensitive to leaf temperature than to air temperature. The response curves of the pathogen to leaf temperature will be presented.
The next step will be the upscaling of the obtained relations to disease dynamics observed on whole plants over a complete epidemic. In a longer term, integration of these relations to epidemiological models of STB is expected to improve the accuracy of models simulating and predicting epidemics, especially in the context of global change.

French population of the wheat pathogen *Mycosphaerella graminicola*: a high level of biological diversity

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The use of different strategies to characterize pathogen populations can be of a great help for a better understanding of plant diseases and an improvement of their control. Here, we describe population genetics and biology of the wheat pathogen *Mycosphaerella graminicola* in France studied by several approaches: mating type assessment, molecular typing and the measure of pathogenicity level and resistance to fungicides. More than 500 isolates of the fungus originating from sixteen localities covering most of the wheat producing areas in France were characterized. Genetic assays disclosed several findings which are consistent with high potential for sexual reproduction within the French population of *M. graminicola*: (i) equal distribution of the two mating types everywhere in the country, (ii) high conservation of the mating type sequences at both nucleotide and population scales, (iii) strong genic and genotypic diversities of the population and (iv) the occurrence of distinct genetic groups within the population as revealed using microsatellite markers and SSCP analysis of actin and *β*-tubulin sequences. The high genetic diversity seems to generate a great diversity at the cytological, biochemical and phenotypic levels within the population, especially regarding pathogenicity determinants such as infection process, cell-wall degrading enzyme production and disease development (leaf necrosis and sporulation). At the field scale, an occurrence of widespread resistance to strobilurin and azole fungicides was disclosed within the population, thereby confirming the high ability of the fungus to overcome chemical treatments. Results reported here indicate that *M. graminicola* possesses a potentially high biological diversity comparing to other plant pathogenic fungi probably conferred by its frequent sexual recombination in the field. We therefore established at different levels the high ability of this plant pathogen to undergo various and efficient adaptations to environmental changes.

Two years of spatiotemporal monitoring of *Mycosphaerella graminicola* airborne inoculum in Belgium

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Two types of dissemination propagules play a role in the epidemiology of Septoria tritici blotch (STB). Airborne ascospores are the major source of primary infections in autumn and winter, whereas the development of the epidemic in spring and summer is thought to be driven by splash-dispersed pycnidiospores. Recent observations in various countries, however, have shown that the airborne inoculum of *Mycosphaerella graminicola* can be produced all year round. In order to understand the role of airborne inoculum in STB epidemics in Belgium, a monitoring technique involving 7-day recording Burkard spore traps and real-time PCR was developed to quantify the total daily airborne inoculum at five locations in the Walloon region in Belgium over 2 years, from April 2009 to April 2011. Four traps were placed in wheat fields and a fifth trap was placed on the roof of a 25 m high building. The reliability of the quantification technique and the homogeneity of the distribution of the STB airborne inoculum at the field scale were controlled in preliminary assays. STB airborne inoculum was detected throughout both years at each of the five locations. Lower but sometimes important quantities were detected in the air above the building, indicating the possibility of long-distance transport. Differences in airborne inoculum quantities in fields were also observed at the network scale and might be linked to the STB level. From the seedling to stem extension stages, there were frequent peaks of detection at each site. The quantities trapped were correlated with the severity of the disease the previous year. Significant quantities of the airborne inoculum were also trapped between the cereal stem elongation and heading stages, suggesting the possible influence of this type of aerial inoculum on STB incidence on the upper leaves in spring and summer. The highest detections, however, generally occurred later, between heading and harvesting, especially in 2009 when disease pressure was high, supporting significant production of pseudothecia during that period. These results illustrate the presence of airborne inoculum all year round, suggesting a possible effect of *M. graminicola* airborne inoculum on STB epidemics throughout the growing season.

Septoria tritici blotch of wheat in Morocco: current status and perspective

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Septoria tritici leaf blotch induced by the heterothallic ascomycete *Mycosphaerella graminicola* is found in most wheat growing areas of the world and is one of the most damaging diseases on wheat in Morocco. It occurs endemically across all wheat growing areas and it is found on the totality of inspected fields of both bread and durum wheat. Its severity ranged from 30 to 100%. In some highly infected fields, yield loss was estimated to be arround 50%. Despite the big effort made to breed for resistance to this disease, national commercial cultivars need further improvement for resistance. To reach such goal, a deep knowledge of the pathogen is a prerequisite. To do so, a project to study the genetic diversity of the population of *Mycosphaerella graminicola* was launched in collaboration with ISA-Lille within a frame of PRAD project. The output of this cooperation is the detection for the first time of the presence of both mating types (MAT1-1 and MAT1-2) of the wheat pathogen *Mycosphaerella graminicola* even on the same leaf lesion and with the same frequency. The presence of the two mating types that offers then a suitable genetic condition for *M. graminicola* to occur sexual reproduction, and the even distribution of early infection in the field that are likely induced by ascospores might be considered as indirect proof of the presence of sexual bodies (pseudothecia) of this pathogen. Accordingly, searching and quantification of pseudothecia in the field will be carried out. Moreover and in order to ensure the durability of resistance of the host, fungicides are to be used in areas where Septoria tritici blotch is known to occur in order to avoid yield losses for susceptible cultivars and to avoid the build up of inoculum levels and consequently to reduce the disease pressure and the risk of breakdown of resistance. However, the pathogen evolves to breakdown the host resistance but also to breakdown the effectiveness of fungicides. Regarding the latter issue, we highlighted for the first time the occurrence of strobilurin-resistant isolates of *M. graminicola* in Morocco. Further genetic investigations will determine if the resistant isolates emerged independently in Morocco or traveled by wind-migration from Europe.

Oral Presentations

Session 3

Pathogen Genetics and Genomics

Peculiar chromosomal evolutionary processes in filamentous fungi, RIP, lateral gene transfer, sectional gene loss and mesosynteny, are amplified in dothideomycetes

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Comparisons of fungal genomes have highlighted four processes which seem to be prevalent and even unique in filamentous fungi. The best known is RIP, a genome defence mechanism that mutates copies of repeated sequences during meiosis. We have developed semi-automated methods of detecting RIP in genome assemblies and of predicting the progenitor sequences. The acquisition of genome sequences of multiple isolates of *Stagonospora nodorum*, has revealed patterns of gene conservation within a species. Extraordinary numbers of genes are unique to each isolate. Furthermore the distribution of "missing" genes is not random. Rather many runs of adjacent genes are missing - a phenomenom we call sectional gene loss. Thirdly comparison of gene content between species suggests that many genes have been laterally transferred into and between filamentous species on times scale that range from decades to millions of years. Comparisons between species reveals a novel form of synteny characterised by retained chromosomal gene content, but shuffled gene order and orientation; we call this mesosynteny. Mesosynteny promises to expedite genome finishing. These four processes are prevalent with the Dothideomycetes, a group that includes the most damaging plant pathogens. We will discuss whether these phenomena have causal relationships and whether this group's success as agricultural patterns can be attributed to these peculiar evolutionary processes.

Evolutionary patterns in *Mycosphaerella graminicola* candidate effectors

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Mycosphaerella graminicola causes Septoria tritici blotch (STB) on wheat leaves and is one of the most damaging diseases of wheat. STB control is possible through the use of fungicides and of resistant wheat cultivars. A better understanding of the mechanisms of *M. graminicola* infectious process is necessary to improve the control of STB. It is likely that *M. graminicola* possesses an arsenal of secreted proteins helping the fungus to infect its host plant. Such proteins are called "effectors". Most effectors are small secreted proteins (SSP) without a known enzymatic function, some of which are translocated into plant cells. These proteins interfere with important plant cellular functions, including defence mechanisms. However, some effectors are recognized by plant resistance genes, inducing a rapid defence reaction and interrupting the infection. These effectors correspond to avirulence genes. The bioinformatic analysis of fungal genome sequences allows the rapid identification of genes encoding SSPs. The genome of *M. graminicola* isolate IPO-323 has at least 489 predicted genes coding for SPP smaller than 300 amino acids. We selected a subset of these genes specifically expressed during infection according to EST libraries. We have confirmed the expression of the selected genes by RT-qPCR *in vitro* and *in planta*, during infection time courses of compatible interactions at two different plant developmental stages (first leaf from seedlings and flag leaf from adult plants). Most of these genes were up-regulated at an early phase of the infection, just before the apparition of the symptoms, at both plant developmental stages. We sequenced the alleles from these genes from 20 *M. graminicola* isolates that have different virulence spectra and are genetically distinct according to molecular markers. Most of these genes have particular evolution patterns either with an increased number of adaptive mutations (non-synonymous) compared to neutral evolution (diversifying selection), or with an exceptionally low number of adaptive mutations (purifying selection). In addition, few genes were either deleted or mutated to an inactive allele, in a number of isolates. This work resulted in the identification of several genes coding for SSPs whose expression is highly up-regulated during the infection and that have clear signatures of diversifying or purifying selection. The functional analysis of these genes is underway to validate their role in fungal virulence or avirulence.

The role of cell wall degrading enzymes during the evolution of *Mycosphaerella graminicola*

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Plant cell wall degrading enzymes (PCWDEs) of plant pathogens are receiving increasing interest for their potential to trigger plant defense reactions. In an antagonistic co-evolutionary arms race between host and pathogen, PCWDEs could be under strong selection. In a first population genetic study, we tested the hypothesis that PCWDEs in the fungal wheat pathogen *Mycosphaerella graminicola* have been positively selected by analyzing ratios of non-synonymous and synonymous nucleotide changes in the genes encoding these enzymes. Analyses of five PCWDEs demonstrated that one (ß-xylosidase) has been under strong positive selection and experienced an accelerated rate of evolution. In contrast, PCWDEs in the closest relatives of *M. graminicola* collected from wild grasses did not show evidence for selection or deviation from a molecular clock. Since the genealogical divergence of *M. graminicola* from these latter species coincided with the onset of agriculture, we hypothesize that the recent domestication of the host plant and/or agricultural practices triggered positive selection in ß-xylosidase and that this enzyme played a key role in the emergence of a host-specialized pathogen. Using a second approach based on comparative genomics, we assessed molecular patterns of adaptation and/or selection of all orthologous PCWDEs on a population genomic scale between *M. graminicola* and its closest relatives.

Comparative proteome analysis of *Mycosphaerella graminicola* IPO323 and IPO323*Wor1*

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Mycosphaerella graminicola is a hemibiotrophic plant pathogenic fungus that causes Septoria tritici blotch, a devastating disease on both durum and bread wheat worldwide. Upon infection, the fungus secretes a repertoire of proteins into the apoplast to suppress or evade plant responses. The identification and characterization of such effector proteins provide a better understanding of the *M. graminicola* lifestyle and pathogenesis. Recently, a putative transcription factor (SGE1) that regulates the expression of six effectors in *Fusarium oxysporum* f.sp. *lycopersici* was functionally analysed. We identified a homologue of this gene in the *M. graminicola* genome that we knocked out which resulted in complete loss of pathogenicity and hence we designated this gene as *Mgwor1*. A potential role as regulator of other genes was subsequently studied by a comparative *in vitro* proteome analysis of *M. graminicola* IPO323 and the *Wor1* knock-out strain IPO323*Wor1*. Secreted proteins of the two isolates were obtained from three different liquid minimal media in four biological replication that were subjected to MS/MS. In total, 115 proteins were identified and functionality annotated with 18 unique proteins with no hits in BLAST analyses. Ninety-nine proteins had predicted signal peptides and two proteins were not expressed in the mutant indicating that these are positively controlled by *MgWor1*. Ongoing experiments, including additional expression analyses of 14 unique candidate effectors, will be discussed.

Population genomics of dispensable chromosomes in *Mycosphaerella graminicola*

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Several plant pathogen species were shown to harbour supernumary or dispensable chromosomes. Such chromosomes were thought to be facultative for the completion of certain life cycle stages. The highest known number of dispensable chromosomes was found in *Mycosphaerella graminicola*. In order to understand the evolutionary history of these chromosomes, we aimed to describe the variability in dispensable chromosomes in 96 isolates originating from the United States, Israel, Australia and Switzerland. We designed a dense PCR assay on exon sequences at regular spacing (approximately 100kb) along all eight known dispensable chromosomes. In total, we scored presence-absence of exon sequences in 72 different genes. In contrast to the Dutch reference isolate IPO323, we found a large number of putatively deleted chromosomal sections in all studied isolates. Furthermore, complete chromosomes were likely missing in some isolates. We detected a high variability in putative length of different chromosomes within and among populations. In order to study the inheritance of dispensable chromosomes, we extended the PCR assay to 48 isolates obtained from two different crosses involving four Swiss isolates. We observed Mendelian segregation for most markers. However, in cases where the parental strains carried more than one putative segmental deletion, we frequently observed strong deviations from Mendelian segregation. Furthermore, complete loss of particular chromosomes likely occurred in several progeny. Taken together, our data showed that variation in dispensable chromosomes was dependent on population structure, including for the large segmental deletions we detected. Mating among isolates with different dispensable chromosome contents may be a key factor contributing to the high variability found in these populations.

Comparative pathogenomics of *Septoria tritici*

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Septoria tritici (teleomorph: *Mycosphaerella graminicola*) causes Septoria tritici blotch (STB) of wheat and is a common and damaging disease in all wheat growing regions of southern Australia. Significant pathogenic variation occurs among Australian isolates of *S. tritici* both between wheat growing regions and over time. *Stb* resistance genes in wheat are well characterized however the genetic basis for pathogenic variation in *S. tritici* is poorly understood. A better understanding of the genetic underpinning of pathogenic variation would be of value to wheat STB resistance breeding programs. We used the recently published genome of *Mycosphaerella graminicola* and our own whole genome assemblies of Australian STB screening isolates in a comparative genomics study to examine the genetic basis of pathogenic variation. Isolates ST79.2.1a and ST55 were chosen for this comparative study because of their postulated contrasting Avr genes. The isolate ST79.2.1a is postulated to carry AvrStb1,2,3,7&12,11,15 / avrStb4,6,10 while ST55 is postulated to carry AvrStb1,2,3,11,15 / avrStb4,6,7&12,10. The corresponding resistance genes have been widely used in Australian and global breeding programs making them an important resource. In addition they provide a contrasting study with the reference isolate IPO323 which carries the isolate specific avirulence gene AvrStb6. The whole genomes of isolates ST79.2.1a and ST55 were sequenced using Illumina $GAII_x$ next-generation sequencing, *de novo* assembled and compared to each other and the reference sequence of *M. graminicola*. Repetitive elements were identified and assembled into classes and the gene content was identified using GeneMark-ES. Genome organization, chromosome number, dispensome arrangement, gene content and genetic variation between the three isolates were compared. The primary objective of the study was to identify and compare AvrStb genes. These genes were functionally annotated and mined for SNPs of biological significance. We present here the results of this study.

Correlation between triazole sensitivity and mutations in the CYP51 gene of *Mycosphaerella graminicola* in Flanders

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In the last decades, a slow but steady shift towards reduced sensitivity of *M. graminicola* to sterol 14α-demethylation inhibitors, of which triazole derivatives are most extensively used, has been observed in Europe. This shift is believed to be mainly caused by mutations in the CYP51 gene encoding the 14α-demethylase target protein for these fungicides, whereby some specific mutations could be linked to the use of certain triazoles. To date, about 19 mutations have been reported in CYP51. In this work, about 100 *M. graminicola* field isolates were sampled at 23 sampling plots spread over wheat-growing areas in Flanders (Belgium) and their sensitivity towards different triazoles was analysed. Furthermore, we sequenced the CYP51 gene of these isolates and performed a mutation analysis on them. The results showed that there is a large variability in triazole sensitivity between the isolates, even within one field, which is reflected in a high diversity in CYP51 haplotypes within the *M. graminicola* population in Flanders. Furthermore, the population is dominated by isolates with several point mutations, which were previously associated with increased resistance towards triazoles. Finally, we found haplotypes that haven't been described in literature before, which might indicate cross-country variation in the *M. graminicola* populations.

From genomic analyses of cereal Pleosporales pathogens to reduced disease losses

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The major foliar pathogens of wheat and barley in Australia and in many other areas on the world are necrotrophic fungi from the order Pleosporales. The wheat pathogens are *Stagonospora (syn. Phaeosphaeria or Septoria) nodorum,* causal agent of Stagonospora nodorum blotch and *Pyrenophora tritici-repentis,* cause of tan spot. The barley pathogens are *Pyrenophora teres teres* (net-type net blotch) and *P. teres maculata* (spot-type net blotch). The adoption of minimum tillage practices and climate change are both linked to emergence and increases in these diseases in the last few decades. Our research group initially focussed on *S. nodorum.* Until recently, this pathogen was treated as an undifferentiated species, which employed a poorly understood arsenal of non-specific toxins and cell-wall degrading enzymes to cause disease. Resistance was partial at best, genetic analysis revealed many weak QTL and no molecular markers were in use. Furthermore, fungicide resistance was reported. A combination of studies based on the genome sequence, released in 2005, revolutionised this picture. *S. nodorum* isolates were found to produce a range of small, secreted proteinaceous effectors (NEs, previously called host specific toxins). This differentiated the pathogen into a large number of races. These effectors interact with wheat sensitivity genes and simplify the interaction into a series of major QTL. The amount of disease is rationalised as a function of the number of effectors produced by the infecting pathogen population that match sensitivity genes in the host. The presence of necrotrophic effectors in *S. nodorum* aligned this species with the archetypal producers of NEs *Cochliobolus heterostrophus, Alternaria alternata* and *Pyrenophora tritici-repentis,* all species from within the newly recognised class the Pleosporales. One of the *S. nodorum* effectors was highly similar to a well known NE from *Pyrenophora tritici-repentis, ToxA.* ToxA versions from both species interact with the wheat sensitivity gene *Tsn1*. Purified ToxA is now used by Australian wheat breeders to select cultivars that are insensitive to the effector and thus more resistant to these diseases. The area planted to ToxA-sensitive wheat cultivars has declined by 500,000 ha in the last 5 years, a development that we estimate has already reduced disease losses by A$10m pa. and which promises to grow to A$150m p.a. The key to this approach is to acquire a series of genomic and genetic resources. These are fungal genome sequences, from which effector gene candidates can be identified and host mapping populations, so that effector/sensitivity gene interactions can be mapped. We have acquired initial genome assemblies of both *P. teres* subspecies. Progress in the use of these assemblies to identify and map effector genes will be described.

Oral Presentations

Session 4
Host-Pathogen Interactions

SnTox1, a cysteine-rich necrotrophic effector produced by *Stagonospora nodorum* triggers light-dependent susceptibility of wheat lines harboring *Snn1*

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SnTox1 was the first necrotrophic effector (host selective toxin) shown to be produced by *Stagonospora nodorum*. SnTox1 interacts directly or indirectly with the product of *Snn1*, the wheat gene located on chromosome 1B that confers sensitivity to SnTox1 as well as susceptibility to *S. nodorum* strains expressing SnTox1. The gene (*SnTox1*) encoding SnTox1 has been verified by heterologous expression in *Pichia pastoris* as well as by gene transformation and knockout experiments in *S. nodorum*. *SnTox1* encodes a 117 amino acid protein with the first 17 amino acids predicted as a signal peptide. The mature protein contains 16 cysteine residues. *SnTox1* was present in ~85% of a global collection of *S. nodorum* isolates. The SnTox1-*Snn1* interaction induces an oxidative burst, DNA laddering, and pathogenesis-related gene expression, all hallmarks of defense response. Interestingly, in the absence of light, SnTox1-induced necrosis and disease symptoms are completely blocked. By comparing the infection processes of a GFP-tagged avirulent isolate and the same isolate transformed with *SnTox1*, we conclude that SnTox1 may play a critical role in penetration. This research provides important insights into the molecular basis of the wheat-*S. nodorum* interaction, an emerging model for necrotrophic pathosystems.

Transcriptomics of the wheat interaction with *Septoria tritici* (*Mycosphaerella graminicola*)

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Septoria tritici blotch (STB) is an important disease worldwide. The genome sequence of the pathogen is available, yet its mode of pathogenicity and the means by which host cells are resistant or susceptible are poorly understood. The TWIST project aims to collect large scale transcriptomic data about different types of interactions (resistant/susceptible) between wheat and *Septoria tritici* (*Mycosphaerella graminicola*). Genome-wide Affymetrix expression arrays are being used to detect genes up- or down-regulated from both the pathogen and the host. Infection time courses have been produced from six wheat cultivars carrying different genetic sources of resistance towards STB, including cultivars with partial resistance, and others with major resistance genes (*Stb* genes). Time courses of infection (0-24 dpi) have been harvested from resistant and susceptible interactions on young leaves and adult flag leaves (14-51 dpi). The RT-qPCR analysis of classic wheat defence gene expression was used to select samples for Affymetrix hybridisation. qPCR fungal biomass measurements and cytological observations are available to support the gene expression data. The wheat gene expression data available so far have shown many differential patterns between susceptible and resistant interactions. A far greater change was observed in wheat gene transcription during the susceptible interactions (~11, 900 genes being differentially expressed) than during the resistant interactions (~3, 100 differentially expressed genes). Functional analysis of the pathways up or down regulated in susceptible and resistant interactions will be presented.

Unidentified *Stagonospora nodorum* necrotrophic effectors and host sensitivity genes detected in southeastern U.S. winter wheat region

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Stagonospora nodorum blotch (SNB) is a ubiquitous and sometimes damaging disease of winter wheat in the southeastern U.S. Twenty-four winter wheat cultivars, mostly advanced experimental lines, from 14 southeastern U.S. breeding programs were chosen due to high levels of either resistance or susceptibility to SNB in Eastern U.S. Uniform Septoria Nursery field trials. A total of 39 *S. nodorum* isolates were derived from wheat debris collected in seven states in the southeastern U.S. Culture filtrates were produced from the isolates and infiltrated into seedling leaves of the 24 cultivars, and sensitivity or insensitivity to the filtrates was assessed visually at seven days post-infiltration. Positive controls included necrotrophic effectors produced by *Pichia pastoris* engineered to express *SnToxA*, *SnTox1*, or *SnTox3*, and spring wheat lines known to be sensitive to *S. nodorum* effectors either singly or in combination. Only one of the 24 cultivars possessed a named sensitivity gene (*Snn3*), judging by the *Pichia* controls. Just one and nine of the 39 isolates appeared to produce SnToxA and SnTox3, respectively, according to the culture-filtrate test. Dot-blot analysis revealed that more of the isolates carry named effector genes than were positive in the infiltration experiment. By dot-blot, however, southeastern U.S. frequencies of named necrotrophic effectors were considerably lower than those found previously in a global population of about 800 isolates: 15% of the 39 isolates carried *SnToxA*, 74% carried *SnTox1*, and 39% carried *SnTox3* in the Southeast, compared to 25%, 85%, and 61%, respectively, in the larger international collections. Based on culture filtrates, 41% of the 39 isolates produced as-yet-unidentified effectors, the targets of which are presumably unidentified sensitivity genes in wheat. Additionally, five SNB-susceptible commercial winter wheat cultivars were screened with filtrates from 23 of the *S. nodorum* isolates originating from four states, along with relevant controls, and only one of those cultivars displayed sensitivity (to SnTox1 and one or more unidentified NEs). The interaction of winter wheat with the *S. nodorum* population in the southeastern U.S. involves a novel set of necrotrophic effectors, probably selected for by the presence of regionally specific host genes for resistance to biotrophic pathogens.

A Blotch on the Landscape: *Mycosphaerella graminicola* and its Interaction with Wheat and Mildew

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The necrotrophic fungal pathogen *Mycosphaerella graminicola* causes Septoria tritici blotch, the most important foliar disease of wheat in Europe. During a compatible interaction, host responses to infection allow the development of necrosis as a form of programmed cell death (PCD) which appears to aid infection rather than hindering it. This contrasts with many host-pathogen interactions involving biotrophic pathogens in which there is a hypersensitive response during incompatible interactions. In comparisons of compatible and incompatible interactions of wheat and *M. graminicola*, genes associated with defence against biotrophic pathogens are expressed in susceptible wheat varieties in response to infection by virulent *M. graminicola* isolates. The interaction of two pathogens infecting the same leaf is a significant issue for disease control in agriculture but is currently poorly understood. The hypothesis that responses to *M. graminicola*, a necrotroph, affect the ability of the plant to respond to biotrophs has been tested. *Blumeria graminis* f. sp. *tritici* causes powdery mildew, another potentially serious disease of wheat. We have developed a method for dual inoculation of wheat leaves with both Septoria and mildew. Leaves initially inoculated with virulent *M. graminicola* and subsequently inoculated with a normally virulent isolate of *B. graminis* had substantially reduced mildew. The very low frequency of colony formation was not the result of reduced success of infection or early development of *B. graminis* spores on leaves inoculated with virulent *M. graminicola*. Genes upregulated during infection with *M. graminicola* potentially inhibit the ability of *B. graminis* to develop on the wheat leaf.

MgWor1 is involved in conidia formation, hyphal morphogenesis and pathogenicity of the fungal wheat pathogen *Mycosphaerella graminicola*

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Mycosphaerella graminicola causes the major wheat disease Septoria tritici blotch in durum and bread wheat especially in regions with high rainfall. Despite its tremendous economic importance the molecular mechanisms controlling the interaction with the host poorly understood. Similar to many other plant pathogens *M. graminicola* produces a wide array of secreted proteins during plant colonization that interfere with host responses. In attempt to gain insight in the regulation of pathogenicity we identified a single copy homologue of the *Wor1* transcription factor in *Candida albicans* that is called *SGE1* in *Fusarium oxysporum* f.sp. *lycopersici* where it regulates effector secretion. We deleted the *M. graminicola* homologue and characterized the mutant. Microscopic observation showed that the mutants do not conidiate and hyphal cells walls are balloon shaped, probably due to cell wall perturbations. Moreover, these strains are dramatically compromised in pathogenicity. We designated the *Wor1* homologue in *M. graminicola MgWor1* and conclude that it is an important pathogenicity factor. Additional proteome studies indicate that *MgWor1* also regulates the expression of specific small secreted proteins that may act as effectors. Ongoing work, including complementation with *SGE1*, will be presented.

Oral Presentations

Session 5

Cultural Measures and Disease Management

Control of Septoria tritici blotch – Aiming at an IPM approach

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Septoria tritici blotch (*Mycosphaerella graminicola*) is seen as the most yield reducing wheat disease in Denmark. Depending on the season and cultivar grown yield reduction due to the disease varies between 5 and 15 dt/ha. Several commonly grown cultivars provide good resistance to Septoria tritici blotch but even so control relies in most seasons on application of triazole-based fungicides using one or two treatments. Precipitation from GS 32 onwards is the main factor used for forecasting the risk of attack. The economically optimal fungicide input for control of Septoria tritici blotch under Danish conditions is in the range of 30-75% of an effective product combination applied applied as either a single or a split application. The last 3 seasons have only given low yield responses (5-6 dt/ha) for control of Septoria tritici blotch, and a negative prognosis being better at predicting the actual loss is still lacking. Over the years a slight shift in triazole sensitivity has taken place using EC_{50} values from epoxiconazole as the main reference. Field performance has been found to be quite stable for the triazoles although a decrease in efficacy has been seen since the epoxiconazole products were introduced in 2004. Microtiter testing has shown shifting in sensitivity for both epoxiconazole and prothioconazole. Characterisation of the CYP51 mutations in the populations has shown a dominance of R6 and R7-types in the Danish population. Repeatedly, the efficacy of epoxiconazole has been seen to be slightly superior to that of prothioconazole, although the yield responses have been equal. Introduction of 3 new triazole mixtures (epoxiconazole + metconazole; difenoconazole + propiconazole; tebucoanzole + prothioconazole) have shown only slight or no increase in efficacy compared to epoxiconazole used as a reference. For the mixture epoxiconazoles + metconazole an improvement has been seen, which relates mainly to a total increase in input. The mixture of epoxiconazole + boscalid has shown slightly improved control and also a slightly increased yield response and is currently seen as the most effective solution. Despite widespread strobilurin resistance a low input of the strobilurin pyraclostrobin mixed with certain triazole products has been found to still give a slight improvement of control and yield.

Chemical control of Septoria species by modern fungicides as an important part of an Integrated Pest Management

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Wheat is one of the world's key food pillars along with rice, corn, millet and soybean. From 1997 to 2005 cereal production grew by only 6.3% whereas the world population increased by 10.5%. 2008 and 2009 were the most productive years ever but in 2010 world wheat production was 4% below the previous year. Meanwhile yield production is stagnating in developed countries. It is expected that utilization of wheat will exceed world production in the 2010/11 marketing year. As a result, a reduction of some 6% (or over 32 million tonnes) in carryover stocks will be required to meet consumption needs. This already resulted in a sharp increase in world prices with wheat currently trading around 50% above the previous year's levels. This development has different consequences for societies depending on whether they are net exporters or net importers of wheat. On the one hand, this offers good opportunities to increase income for countries producing more grain than consumed. On the other hand, for countries where 40% or more of income needs to be spent on nutrition and not enough grain can be produced locally, this can have severe socio-economic consequences. Independently from whether people are living in areas with a positive or negative wheat producing balance, it would be beneficial if they could produce a consistently higher yield level. Unfortunately output is endangered in some areas by unpredictable water supply, and even where enough water is available severe outbreaks of disease epidemics can have dramatic and tragic effects. The infections with Septoria species are the main caused for dramatic yield depression in many wheat growing countries. To avoid the dramatic consequences of yield losses of up to 30% possible an integrated pest management program could be a promising approach. Besides breeding for more tolerant or even resistant wheat varieties, the use of modern fungicides for disease control and yield protection offers an important tool to ensure both the production of food and farmers income. In this presentation a general overview on Integrated Pest Management including fungicidal usage is given. It illustrates how far new chemistry is investigated during the registration process to avoid harm to the user, the environment and the consumer of treated crops. In addition, experiences from North and Central European countries on the most efficient way to use fungicides are highlighted. Experience from decades of research performed in various cooperations between crop protection manufacturers and independent organizations under various climatic conditions are available. Important technical principles have global validity. Recent results on the use and value of fungicide applications in North Africa and the Near to Far East as in northern Europe are presented. Finally, the first ideas on how to best introduce fungicides under local conditions will be discussed. This will include a first outline on how to implement IPM measures on the ground with specific emphasis on the use of modern, highly effective cereal fungicides.

Functional characterisation of *CYP51* changes identified in azole resistant isolates of *Mycosphaerella graminicola*

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Azole (imidazole and triazole) fungicides are relied on for the control of Septoria leaf blotch, caused by *Mycosphaerella graminicola*. The dependence on azoles for Septoria leaf blotch control is set continue for the foreseeable future as newly introduced fungicide groups, for example the succinate dehydrogenase inhibitors (SDHIs), are used in mixtures with azoles. Therefore, the recent development of resistance to some azoles and a decrease in sensitivity to others in Western European populations of *M. graminicola*, is the primary threat to control of this disease. We, and others, have identified changes in the coding region of the *CYP51* gene encoding the azole target, the sterol 14α-demethylase, associated with azole resistant phenotypes. The precise impact of these changes on azole fungicide sensitivity and intrinsic protein function was established by heterologous expression in yeast and the results provide a rationale for the sequence in which *CYP51* mutations emerged in Western European populations. We have also demonstrated the effect of recently identified CYP51 variants on sensitivity to the two most effective azoles, epoxiconazole and prothioconazole, and by using a novel approach, developed a model of the *M. graminicola* CYP51 protein that includes important fungal specific regions for which no structural information is available. We shall also present homologous gene replacement studies that provide direct evidence of the effect of changes in *CYP51* coding and regulatory regions on *M. graminicola* isolate azole sensitivity.

Expansion of green leaf area in relation to reduction of Septoria leaf blotch severity: comparison between bread wheat cultivar mixtures and pure stands

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Given the erosion of fungicide efficiency and the breakdown of resistance genes, there is an urgent need to identify alternative farming practices, such as the increase of within-field genetic diversity. Mixtures of wheat cultivars with different resistances to pathogens can gain performance compared to the average of pure stands by reducing severity of diseases such as rusts and powdery mildew of cereals, which are due to air-borne pathogens. However the efficiency of cultivar mixtures for controlling splash-dispersed pathogen such *Mycosphaerella graminicola*, with pycnidiospores being carried by droplets produced by raindrop impacts on lesions, remains poorly documented. On-farm trials have shown that cultivar mixtures with a single fungicide application can slightly reduce septoria leaf blotch epidemics while stabilizing productivity and crop quality. Our aim was to assess how cultivar mixtures can reduce septoria leaf blotch progression as a function of rain events. Field experiments during four years were carried out on small plots, replicated four times, with pure stands and 1:3 mixture of a susceptible and a partially resistant cultivars (with and without fungicide treatment). We assessed weekly the number of sporulating lesions, diseased and green foliar surfaces on the three last leaves which are determinant for grain filling. During the two years with significant disease, the number of sporulating lesions per susceptible plant of the cultivar mixture was greater until 6.3 (2008) and 16.5 (2009) compared to the susceptible pure stand, after a 10 mm cumulative rain. The green leaf area of the susceptible cultivar in mixture was increased by 11% and 20%, respectively, during the 2 years, due to a decrease of 6% and 20% of diseased area, compared to the susceptible pure stand. Cultivar mixtures allowed to delay leaf blotch spreading to save until one green leaf for the susceptible cultivar in mixture compared to the pure stand, at the heading stage. Furthermore, green leaf area of the susceptible cultivar in unsprayed cultivar mixture was intermediate between green leaf area of the sprayed and unsprayed pure stands of the susceptible cultivar.

Experimental strategies to unravel the MDR mechanism in new emergent phenotypes of *M. graminicola* highly resistant to several DMIs

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The mechanism of Multidrug Resistance (MDR) is well described in human pathogens such as those provoking candidiasis and mycosis or in human diseases such as cancer cell lineages. This mechanism is mainly driven by transporters extruding drugs outside the cell. In agriculture, the wheat Septoria tritici blotch fungus *Mycosphaerella graminicola* shows a progressive and continuous increase in its ability to resist sterol 14α-demethylation inhibitors (DMIs). Our last field surveys, based upon phenotype analysis of bulk populations, allowed the detection of strains with highly resistant phenotypes to some or all DMIs [from French, English and Irish populations]. These isolates were first observed at very low frequency but reached 30% of the French populations in 2010. In field isolates weakly or moderately resistant to DMIs *in vitro*, one or several mutations were recorded in the target encoding-gene *Cyp51*. In some field isolates highly resistant to DMIs, cross-resistance with QoIs and SDHIs (two classes of respiratory inhibitors) was also recorded, suggesting a combination of alterations in *Cyp51* and overexpression of drug efflux transporters to be involved in these multidrug resistant (MDR) phenotypes. Finally, some isolates moderately or highly resistant to DMIs harbour an insertion in the *Cyp51* promoter and/or new combinations of already known mutations in the target gene. Our results show that there is (*i*) no obvious correlation between the promoter insertion, the *Cyp51* expression level under azole exposure and the MDR phenotypes; (*ii*) In a ^{14}C radio-labelled DMI accumulation rate experiment using two susceptible strains vs. two MDR strains we specifically demonstrated that the MDR phenotype could be linked to an ATP-dependent azole transport system and (*iii*) We performed crosses between two MDR strains as well as crosses to a sensitive strain. The latest data and analyses will be discussed.

Azole resistance is related to overexpression of the *CYP51* gene in *Mycosphaerella fijiensis*

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Mycosphaerella fijiensis causes the threatening black Sigatoka disease in bananas and plantains. Cavendish cultivars are particularly highly susceptible and disease management is mainly through the application of systemic fungicides, including azoles. However, as expected, their intensive use has favoured the appearance of resistant strains. Previous studies showed several point mutations in the *CYP51* gene close to the putative substrate-binding site that were correlated with resistance to cyproconazole and propiconazole. In the present study we found that strains with very significant resistance phenotypes to azoles have more than one insertion of a 19 bp repeat element in the promoter region of the *CYP51*gene. qRT-PCR analysis revealed that strains with the highest number of repeat insertions overexpress the CYP51 gene. We then developed a PCR-based assay to screen field populations for the presence of the repeat unit and found that populations from regions under high selective pressure have much higher frequencies of strains containing five or more repeat units. The data show that the number of repeats in the promoter and the presence of mutations in the coding region of the *CYP51* gene are clearly related to reduced azole sensitivity in *M. fijiensis.* These studies will provide important information to optimize azole management for black Sigatoka control.

Oral Presentations

Session 6

Tan spot and other Septoria Like Diseases

Studies on host-pathogen interactions in tan spot [*Pyrenophora tritici-repentis*] of wheat

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Tan spot, caused by the fungus *Pyrenophora tritici-repentis*, is an important foliar disease of wheat. Eight races of *P. tritici-repentis* have been identified to date, based on their pathogenicity on a host differential set. The development of tan spot results from the action of host-specific toxins, namely Ptr ToxA, Ptr ToxB and Ptr ToxC, which are differentially produced by isolates of the fungus. The long-term aim of our research program is to obtain a comprehensive understanding of the wheat/*P. tritici-repentis* interaction, particularly as it pertains to pathogenicity, virulence and symptom development. Much of our attention has been focused on Ptr ToxB, a 6.6 kDa protein that induces chlorosis on sensitive wheat genotypes and is encoded by the ToxB gene. Copies of this gene are present in different numbers in isolates of *P. tritici-repentis*, with copy number correlated with the degree of pathogenicity on Ptr ToxB-sensitive hosts. Silencing of ToxB in a wild-type isolate through a sense and anti-sense mediated silencing mechanism revealed that the toxin confers virulence in a dosage-dependent manner, suggesting that Ptr ToxB functions not only as a pathogenicity factor essential for infection by *P. tritici-repentis*, but also as a virulence factor contributing quantitatively to symptom severity. In sensitive host leaves, Ptr ToxB causes a rapid decline in photosynthesis, which is accompanied by significant changes in the abundance of proteins involved in the light reactions of photosynthesis, the Calvin cycle, and the stress/defense response. These findings suggest that a Ptr ToxB-mediated disruption of photosynthetic processes results in the generation of reactive oxygen species, leading to chlorophyll photooxidation and, ultimately, foliar chlorosis. Collectively, the information obtained from studies on tan spot of wheat will not only further our understanding of pathogenicity in *P. tritici-repentis*, but may also provide insights into other plant/fungal pathosystems involving host-specific toxins.

Quantitative variation in activity of ToxA haplotypes from *Stagonospora nodorum* and *Pyrenophora tritici-repentis* refines the distinction between biotrophic and necrotrophic interactions

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The parallels between host-specific toxins produced by necrotrophic pathogens (we now prefer to call them necrotrophic effectors (NEs)) and avirulence gene products produced by biotrophs (we suggest the name biotrophic effectors or BEs) have accumulated over the last decade. The effectors produced by both classes of pathogen operate in a species- and cultivar-specific manner and produce reactions in the host that are operationally very similar. In at least three cases, we now know that the host partner (the direct or indirect receptor) for NEs is - like in BEs - an NBS-LRR gene. Nonetheless there is a clear functional difference; recognition of a BE leads to resistance; recognition of the NE leads to virulence. Overall, resistance in biotrophic interactions tends to be qualitative because recognition of a single effector is sufficient to induce resistance. In contrast, resistance in necrotrophic interactions tends to be quantitative; this has assumed to be because multiple effectors interact with multiple receptors and each positive interaction acts quasi-additively to produce the virulence phenotype. High dN/dS ratios have been observed in many BE and R-genes in biotrophic interactions and this has been cited as evidence of diversifying selection associated with qualitative interactions leading to a co-evolutionary arms race. This paper discusses and refines the parallels between NEs and BEs in the light of new data about ToxA. ToxA is a proteinaceous NE produced by two wheat pathogens, *Stagonospora nodorum* and *Pyrenophora tritici-repentis.* Both pathogens produce several other NEs and resistance is quantitatively inherited. ToxA makes a significant contribution to virulence in interactions of both species with wheat cultivars that carry the NBS-LRR gene *Tsn1*. Seven mature versions of the ToxA protein are encoded by different *ToxA* genes in *S. nodorum* populations while a single version exists in most isolates of *P. tritici-repentis.* The genes exhibit an elevated dN/dS ratio. known sensitive alleles of the gene in bread wheats encode an identical protein. We expressed and purified 8 versions of ToxA. Circular dichroism spectra indicated that all versions were structurally intact and have indistinguishable secondary structural features. We expect that each variant has a similar tertiary structure. All versions induced necrosis when introduced into any *Tsn1* wheat line. However, we observed quantitative variation in effector

activity for the different ToxA variants. The least active version was the one present in isolates of *P. tritici-repentis*. Different wheat lines carrying identical *Tsn1* alleles varied in sensitivity to ToxA. The presence of diversifying selection is often indicative of direct protein-protein effector/receptor interactions leading to a co-evolutionary arms race. If diversifying selection led to the observed *ToxA* diversity, then given that all *Tsn1* alleles are identical, we predict that the gene or genes encoding wheat proteins that interact with ToxA and with the *Tsn1* gene product will also show diversifying selection. An alternative explanation for the observed diversity is directional selection that has favoured *ToxA* alleles encoding higher virulence in regions where *Tsn1* wheat is common. In either scenario, our results indicate that subtle differences in effector alleles may underlie quantitative differences in virulence in gene-for-gene systems.

First report of the sexual stage of *Pyrenophora tritici-repentis* in Iran

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Recently tan spot disease of wheat caused by *Pyrenophora tritici-repentis* has become very important disease in north parts of Iran and causes severe yield loss. Although the anamorph of the fungus has been reported previously on infected leaves from Mazandaran province (Forotan *et al*., 1996), but there is no report on mass production of conidia in laboratory and the telemorph of the fungus in Iran. Infected leaves were collected from wheat fields in Golestan and Mazandaran provinces and were investigated in laboratory and greenhouse conditions. The results showed that after seven days the colonies of the fungal mycelium in V8-CaCo3 medium was dark gray color, conidiophores swollen at the base, yellow to brown color, 7-8 µm thick and 100-300 µm long, conidia were yellow, with 5-7 pseudosepta, 14-18 × 100-200 µm and the basal cells were conical. 5-mm plug taken from an active colony margin of a young culture grown on V8-CaCo3 were used to inoculate 2%WA medium containing wheat leaves and these media were kept in special treatment including 12 hours photoperiod of white fluorescent and near UV light at 22 ºC. After two weeks, dark colored pseudothecia with 200-250 µm size, were produced on leaf tissues, Ascospores were oval shape with three transverse and one longitudinal septa, and 16-20 × 40-50 µm size. Based on morphological characters the fungus was identified as *Pyrenophora tritici-repentis*. Pathogenicity test was carried out on susceptible cvs, Bolani and Tajan with the suspension of ascospores with concentration of 10^6 at 2-3 leaf stage. Inoculated seedlings were kept under black plastic bags for 48 hours at 22 ºC with the photopriod of 16 hours. Plastic bags were removed after that and inoculated seedlings were placed in greenhouse at 22 ºC with the photopriod of 16 hours. Symptoms of the disease including distinct necrotic and chlorotic lesions were observed after six days. The fungus was isolated from the infected leaf tissues. This is the first report of sexual stage of *Pyrenophora tritici-repentis* from Iran.

Oral Presentations

Session 7
Host Genetics and Resistance Breeding

Association genetics of resistance to Septoria tritici blotch in north-west European wheat cultivars and breeding lines, and implications for resistance breeding

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Septoria tritici blotch, caused by *Mycosphaerella graminicola*, first became a commercially important disease of wheat in Europe around 1980. A genome-wide association mapping analysis was conducted to investigate the genetics of resistance to Septoria in germplasm used in breeding in the United Kingdom from the 19th and 20th centuries. The entire population of 225 wheat cultivars and breeding lines was tested with Diversity Array Techology (DArT) markers, while a subset was also tested with simple-sequence repeat (SSR, microsatellite) markers. Two methods of estimating kinship were used, from published pedigrees and from sharing of alleles at unlinked loci. The set of lines tested formed a single population, with no evidence for sub-division, owing to the presence of several common ancestors of large parts of the pedigree. Use of both types of kinship estimate led to similar postulations of quantitative trait loci (QTL) controlling Septoria resistance on 11 of the 21 chromosomes in wheat. Abundant genetic variation in partial resistance to Septoria in UK wheat cultivars was therefore detected. This may be exploited in breeding to improve Septoria resistance, for example by selecting lines which combine genes from different lineages and thus have greater resistance than either parent. Calculation of best linear unbiased predictions (BLUP) of susceptibility to Septoria allowed identification of lineages of the UK winter wheat pedigree through which four chromosome regions affecting significant proportions of resistance have been transmitted as well as one such chromosome region in spring wheat. It is very likely that genes which increased susceptibility (or reduced resistance) to Septoria were introduced inadvertently into wheat breeding programmes from cultivars used to increase yield, rust resistance and eyespot resistance between the 1950s and 1980s. This implies that there is a significant potential cost to using non-adapted material in plant breeding, in the form of an accidental increase in susceptibility to a previously insignificant disease.

QTL analysis of resistance to three isolates of *Mycosphaerella graminicola* in wheat

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Effective management of resistance to *M. graminicola* became a critical issue in regions were Septoria tritici blotch (STB) is prevalent disease of wheat. Resistance to the disease is considered as isolate-specific or quantitative. Polish wheat cultivar Liwilla express resistance to STB both at seedling stage and under field conditions. In order to map loci underlying resistance to the disease, a doubled haploid (DH) population was developed from the cross between Liwilla and susceptible cultivar Begra. This population was evaluated under controlled environment on a second leaf. In experiments single isolates of *M. graminicola* IPO92006, IPO86036 and IPO323 were used. Two disease parameters were scored: percentage of necrotic leaf area (N) and percentage of leaf area covered with pycnidia (P). Mapping population (Liwilla × Begra) were used to construct molecular map of wheat comprising DArT (Diversity Arrays Technology) and microsatellite markers. A QTL (Quantitative Trait Loci) analysis was performed using multiple QTL model (MapQTL 5.0) and several QRLs (Quantitative Resistance Loci) associated with measured disease parameters were detected. QRLs associated with low N value were identified on chromosomes 1B (isolate IPO92006), 2B (isolate IPO86036), 3B (isolate IPO86036), 6B (isolate IPO323), 3A (isolate IPO323) and 6A (isolate IPO323). QTLs detected on 2B, 3A and 6A overlapped with quantitative resistance loci responsible for low P. The percentage of phenotypic variance explained by a single QTL ranged from 8.8 to 40% for N and from 6.5 to 43.1% for P.

Using sources of intermediate resistance to identify quantitative resistance loci to *Septoria tritici*

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Inheritance and genetic studies of resistance to Septoria tritici blotch (STB) have mainly focused on the dissection of highly resistant source material. As a result, 15 isolate specific resistance loci and at least 20 QTL have been described over the last decade. These studies will continue to be important for resistance gene discovery. However, qualitative resistance is unlikely to provide long-term durability to breeding programs. To complement these major genes, studies are required which address our understanding of intermediate resistance. In this present study, we have examined the inheritance of resistance to STB of two commonly used sources of intermediate resistance in Australian breeding programs, Janz and Diamondbird. The study is based on a doubled haploid (DH) population of 195 lines, multi-isolate screening of the parents and the DH population plus supporting analysis from breeding populations. The isolates used to screen the DH population were 79.2.1a (postulated AvrStb1, 2, 3, 7 & 12, 11, 15/avrStb4, 6, 10) and ST55 (postulated AvrStb1, 2, 3, 11, 15/avrStb4, 6, 7 & 12, 10) which originate from south east Australia. Parental screening with six isolates revealed intermediate reactions and that neither parent carries any of the previously identified resistance genes. The DH population was mapped with 531 genome wide SSR and DArT markers. Phenotyping of the population revealed a continuous distribution of disease response with significant transgressive segregation towards both increased resistance and susceptibility. The identified QTL will be discussed in detail.

Efficacy and mapping of resistance to *Mycosphaerella graminicola* in wheat

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Septoria tritici blotch (STB), caused by the ascomycete *Mycosphaerella graminicola*, is one of the most devastating foliar diseases of wheat. We screened five synthetic hexaploid wheats (SHs), 13 wheat varieties that represent the differential set of cultivars and two susceptible checks with a global set of 20 isolates and discovered exceptionally broad STB resistance in SHs. Subsequent development and analyses of recombinant inbred lines (RILs) from a cross between the SH M3 and the highly susceptible bread wheat cv. Kulm revealed two novel resistance loci on chromosomes 3D and 5A. The 3D resistance was expressed in the seedling and adult plant stages, and it controlled necrosis (*N*) and pycnidia (*P*) development as well as the latency periods of these parameters. This gene, which is closely linked to the microsatellite marker *Xgwm494*, was designated *Stb16* and explained from 41 to 71% of the phenotypic variation at seedling stage and 28 to 31% in mature plants. The resistance locus on chromosome 5A was specifically expressed in the adult plant stage, associated with SSR marker *Xhbg247*, and explained 12 to 32% of the variation in disease. This quantitative trait locus (QTL) was designated *Stb17q*, and is the first QTL for adult plant resistance to *M. graminicola* to be identified. Our results confirm that common wheat progenitors might be a rich source of new *Stb* resistance genes/QTLs that can be deployed in commercial breeding programs. A screening of the French bread wheat cultivars Apache and Balance with 30 *M. graminicola* isolates revealed a pattern of resistant responses that suggested the presence of new genes for STB resistance. Quantitative trait loci (QTL) analysis of a doubled haploid (DH) population with five *M. graminicola* isolates in the seedling stage identified four QTLs on chromosomes 3AS, 1BS, 6DS and 7DS and occasionally on 7DL. The QTL on chromosome 6DS flanked by SSR markers *Xgpw5176* and *Xgpw3087* is a novel QTL that now can be designated as *Stb18*. The QTLs on chromosomes 3AS and 1BS most likely represent *Stb6* and *Stb11*, respectively, and the QTLs on chromosome 7DS are most probably identical with *Stb4* and *Stb5*. However, the QTL identified on chromosome 7DL is expected to be a new *Stb* gene that still needs further characterization. Multiple isolates were used and show that not all isolates identify all QTLs, which clearly demonstrates the specificity in the *M. graminicola*-wheat pathosystem. QTL analyses were performed with various disease parameters. The development of asexual fructifications (pycnidia) in the characteristic necrotic blotches of STB, designated as parameter *P*, identified the maximum number of QTLs. All other parameters identified fewer but not different QTLs. The segregation of multiple QTLs in the Apache/Balance DH population enabled the identification of DH lines with single QTLs and multiple QTL combinations. Analyses of the marker data of these DH lines clearly demonstrated the positive effect of pyramiding QTLs to broaden resistance spectra as well as epistatic and additive interactions between these QTLs. Phenotyping of the Apache/Balance DH population in the field confirmed the presence of the QTLs that were identified in the seedling stage, but *Stb18* was inconsistently expressed and might be particularly effective in

young plants. In contrast, an additional QTL for STB resistance was identified on chromosome 2DS that is exclusively and consistently expressed in mature plants over locations and time, but it was also strongly related with earliness, tallness as well as resistance to Fusarium Head Blight. Although to date no *Stb* gene has been reported on chromosome 2D, the data provide evidence that this QTL is only indirectly related to STB resistance. This study shows that detailed genetic analysis of contemporary commercial bread wheat cultivars can unveil novel *Stb* genes that can be readily applied in marker-assisted breeding programs.

Strategies for genetic enhancement of resistance to *Stagonospora nodorum* and *Pyrenophora tritici-repentis* in wheat

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In Western Australia Stagonospora nodorum blotch and tan spot frequently occur together and present important resistance breeding targets. Research has focused on understanding the genetic control and deployment of resistance to the two diseases in relevant wheat genetic backgrounds. Quantitative trait loci (QTL) for *Stagonospora nodorum* flag leaf resistance on chromosome 1BS, 2AS, 2DL and 5BL and glume resistance on 4BL and 2DL have been consistently identified in at least 2 environments and account for 10-40% of the total phenotypic variation in various spring and winter genotypes. It is yet inconclusive whether adult plant resistance in any winter or spring wheat genotype is associated with effector based insensitivity loci. The chromosomal map position of markers associated with flag leaf resistance QTL on 1BS, 2DL and 2AS indicates that they are unlikely to be associated with known host-toxin insensitivity loci. QTL on chromosome 5BL from a spring wheat cultivar EGA Blanco had a highly significant association with markers *fcp001* and *fcp620* based on disease evaluation in 2007 and, therefore, is likely to be associated with *tsn1*-ToxA insensitivity for flag leaf resistance. However, *fcp001* and *fcp620* were not associated with a QTL detected based on disease evaluation in 2008, indicating two linked QTL for flag leaf resistance with multiple genes residing on 5BL in EGA Blanco. The deployment of various QTL for *S. nodorum* resistance in susceptible genetic backgrounds relevant to Western Australian environments has been successfully achieved and the effects of different QTL for improving resistance to stagonospora nodorum blotch is currently being investigated through phenotypic evaluation. Research on tan spot has mainly focused on identifying genes other than *tsn1* (5BL) which is currently the only known gene used in Australian breeding programs. So far, three mapping populations have been assessed at different growth stages and in multiple environments. Transgressive segregation was observed for seedling, tillering and adult plant response to tan spot in various populations. Disease correlations between growth stages and between environments were moderate (r = 0.4 to 0.5). Various QTL for tan spot resistance have been identified on chromosomes 1A, 2A, 4B, 6B and 7B. Efforts are now focused on developing a series of fixed lines, each carrying tan spot resistance genes from various sources, in elite Australian backgrounds to generate potential parental stocks enriched for resistance.

Developing and sharing tools to bring benefits of resistance to *Mycosphaerella graminicola* to wheat growers in France: from breeding to cultivar registration and extension

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In France, Septoria tritici blotch (STB) causes an average of 1.5 t/ha yield loss in the absence of fungicides, with incidental peaks reaching over 4 t/ha in conducive environments. Disease resistance reduces these losses and contributes to reduction in fungicide use. Building on existing projects between academic research and breeding companies, a collaborative three-year project financed by the wheat growing industry (Fonds de Soutien à l'Obtention Végétale) was set up to developand improve common tools all along the "cultivar lifetime continuum", from breeding to registration and advice, to allow growers to benefit fully from recent research on STB resistance. Tools span from improved quantification of fungal biomass by qPCR, as well as novel and improved molecular and phenotypic tools for the characterization of STB populations and of resistances of wheat cultivars to STB, in field and controlled conditions. An extensive survey of French STB isolates has been collected, and will be phenotyped and genotyped. In parallel, 60 French cultivars were phenotypically characterized for known STB resistance genes. Published markers linked to these genes were tested on the same set of cultivars, highlighting both prospects and limits of this existing knowledge for French growers. Tools were also refined for the study of quantitative resistance. Finally, methodologies for evaluating field resistance are refined and harmonized between partners. The perspectives of applying these tools to bring better STB resistance into the field are discussed.

Characterization of CIMMYT germplasm for resistance to Septoria diseases of wheat

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The leaf spotting disease complex is a major biotic constrain in enhancing grain production in the major wheat growing regions. This complex of diseases include two major Septoria diseases: Septoria tritici blotch incited by the fungus *Mycosphaerella graminicola* (anamorph: *Septoria tritici* Rob. ex Desm.) and Stagonospora nodorum blotch caused by *Phaeosphaeria nodorum* (anamorph: *Stagonospora nodorum* (Berk.) Castellani & Germano). Both diseases cause serious losses in quality and quantity of grain produced mainly in the high-rainfall areas of wheat production. Epidemics of Septoria tritici blotch and Septoria nodorum blotch of wheat are associated with favorable weather conditions like frequent rains and moderate temperatures, conservation agriculture practices, and wheat monoculture involving cultivation of susceptible cultivars. International Maize and Wheat Improvement Center (CIMMYT), Mexico initiated major efforts to mitigate the threat of Septoria tritici blotch in early 1970s and semi-dwarf wheat germplasm with adequate resistance were developed and distributed globally. Current efforts include screening of wheat germplasm, identification and characterization of new sources of resistance, mapping genes conferring resistance and incorporation of resistance into new, high-yielding backgrounds. Screening trials of elite CIMMYT germplasm from Irrigated, Rainfed and Wide-Crosses Breeding programs reveal high level of resistance to both the Septoria diseases. These germplasm have diverse genetic make-up and the resistance is likely broad based. Genetic and molecular analysis of elite breeding materials is in progress to identify novel resistance genes. Additional efforts are in place to develop superior wheat cultivars with durable Septoria resistance. The International Septoria Nursery (ISEPTON) has been re-established and advanced breeding lines with diverse genetic make-up showing promising agronomic, quality and Septoria resistance are distributed worldwide to be utilized by wheat breeding programs in development of superior wheat cultivars.

Screening potential donors of resistance to Septoria tritici blotch (*Mycosphaerella graminicola*)

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In the period 2008-2010, field tests were conducted on a set of spring cereal crops consisting of 20 cultivars of spring wheat (*Triticum aestivum* L.), 10 lines of tritordeum (×*Tritordeum* Ascherson et Graebner), a line of haynaldoticum (×*Haynaldoticum sardoum* Meletti et Onnis) named Denti de Cani C.P. and selected synthetic wheat CIGM93.266. Six different isolates of Septoria tritici blotch causing fungus (*Mycosphaerella graminicola* Fuckel/ J. Schröt.) were used for artificial infection. Responses of genotypes to three isolates were tested each year. The isolates used in 2009 and 2010 were identical. A variant without artificial infection was a check. The aim was to select potential resistance donors. The infection level was measured as a percentage of the disease on four upper leaves of 15 stems per genotype. The infection was largely affected by weather conditions over the season and virulence of the isolate used. Average disease severity in spring wheat cultivars was 4.11% (check 0.49%), tritordeum 0.03% (check 0.02%), synthetic wheat CIGM93.266 0.21% (check 0%) and Denti de Cani C.P. 0.08% (check 0 %). The results confirmed a high level of resistance in tritordeum, haynaldoticum and synthetic wheat. Preliminary results indicate that we have resistant forms of winter wheat possessing the resistance transferred from tritordeum, where wild barley (*Hordeum chilense* Roemer et Schultes) is considered as a progenitor of the non-host type of resistance to this disease.

Lancero INIA [BEZ/4/II.5072/Y54//NOR10B/3/MD/IB] the perfect alternative wheat cv. for Chile but susceptible to the *Mycosphaerella graminicola – Septoria tritici* pathosystem

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Lancero INIA was one of the last releases of the late plant breeder Mrs. Lilian Aguayo from INIA Chile. The cultivar was introduce on 1982 and immediately accepted by famer who found it adjust for early and late planting. It was withdraw from breeder support on 1998, after 16 years of successful cultivation, because it become completely susceptible to Septoria Leaf Blotch [SLB]. With chemical support still is been use by a few farmers. It has an alternative type of growth joining the required lateness for a crop that need to survive heavy rainfall, fast grain filling under drought but not necessarily possess winter hardiness. Lancero had been use for epidemiological, breeding and chemical studies for several years. Every year the phenology of the fungus was monitored on Lancero beginning with the maturity of the pseudothecia and ascospores discharge of the teleomorph [April – May] on infected non chemical sprayed stubble from previous season, the appearance of pycnidia [June – October] from the lower to the upper leaves of the plant depending of the amount of rainfall, and seldom the occurrence of pycnidia on head [January]. Summer months maintain the stubble intact and only the first rains on March – April will trigger the teleomorph formation to restart the cycle. The repeatability of the results using Lancero as susceptible control on breeding nurseries help to select every year new combinations of genotypes with low SLB scores. Ten chemical control experiments on Lancero realized between 1998 to 2009 had been very useful to understand the impact of the disease on each crop season. Experiments were carried out on randomize block design, 9 to 12 chemical treatment, four replicates, experimental unit of six two meter rows 0,2 m spaced. Control plots without chemical protection yield 6.08 t/ha meanwhile the best treatment reach 9.05 t/ha (** $p<0.05$) and disease record dropped from 93 vs 45 on double digit Eyal/Saari Prescott [DD] scale respectively. The most significant response to fungicide [+ 49 %] occur on 2002 crop cycle when annual rainfall reached 1630 mm, 45 % greater than a normal year. SLB of the untreated control climb to 9_30 score, DD scale and became the only record we have SLB was able to form pycnidia on the awns and glumes. Lancero is an adequate genotype for SLB studies.

A yield penalty of Septoria resistance – and can it be overcome?

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Since the emergence of Septoria tritici blotch, caused by *Mycosphaerella graminicola*, around 1980 as the major disease of wheat in Europe, it has been a significant challenge for breeders to release varieties which combine very good resistance to Septoria with the high yields expected in modern agriculture. An association genetics analysis showed that while yield and Septoria resistance both advanced in wheat varieties bred for the UK in the latter part of the 20th century, selection for increasing yield inhibited the advancement of Septoria resistance. It was also evident, however, that there must be genes which increase yield but do not depress Septoria resistance, or *vice-versa*. The yield penalty of Septoria resistance was analysed further in a recombinant doubled-haploid (DH) population of 130 lines derived from the cross Spark x Rialto. Two QTLs were identified as making significant contributions to Septoria resistance, one on chromosome 3A, at or near the *Stb6* locus, and one on chromosome 4D, close to the *Rht-D1b* (formerly *Rht2*) locus. The effect of *Rht-D1b* on Septoria was complex; while the semi-dwarfing allele was associated with reduced Septoria disease, consistent with reduced plant stature being a risk factor for Septoria, lines with this allele tended to have less Septoria on flag leaves than plants of the same height which had the wild-type 'tall' allele. The relationship between susceptibility to Septoria and yield potential depended on the fertility of the trial site. Septoria data was obtained from disease trials in England in 2006 and 2008 while yield data had been obtained from an earlier series of trials in the UK, France and Germany in diseases, including Septoria, were controlled. Septoria-susceptibility was associated with high yields at sites at which the mean yield of Spark x Rialto DH lines was high but with low yield at sites with low mean yields. These data support the hypothesis that there is a yield penalty of having Septoria resistance when wheat is grown in modern, intensive farming conditions. It should be possible, however, for breeders to select lines which combine high yields with high levels of durable, partial resistance to Septoria.

Inheritance of total resistance to *Mycosphaerella graminicola* isolate Tun6 in a local durum wheat 'Agili'

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High resistance to virulent Tunisian isolate of the ascomycete fungus *Mycosphaerella graminicola*, which causes Septoria tritici blotch of wheat, has been detected in old local durum wheat 'Agili'. High yield durum wheat cvs. 'Karim' and 'Khiar', susceptible to the virulent isolate 'Tun6' were crossed with the resistant 'Agili'. In both F_2 populations, a segregation 3:1 (resistant/susceptible) was observed after inoculation in the field with the virulent pathotype 'Tun6' at seedling and adult stage, indicating that the resistance is controlled by a single dominant gene. This was supported by F_2-derived F_3 families segregating in a 1:2:1 (homozygous resistant/segregating/homozygous susceptible) ratio and F6 progeny segregating 1:1 (homozygous resistant/homozygous susceptible). These data are consistent with a gene-for-gene interaction in the wheat-*M. graminicola* pathosystem and provided evidence that qualitative resistance to Seproria tritici blotch exist in durum wheat.
Analysis of the polymorphism between the resistant ('Agili') and the susceptible ('Khiar' and 'Karim') parents using 19 SSR linked markers to stb genes revealed that only 7 markers were polymorphic. The resistance gene of 'Agili' was not linked to any of these markers in particular with the markers for *Stb2, Stb7* and *Stb12* genes indicating a different source of resistance carried by 'Agili'.

Delivering disease resistant cultivars to enhance sustainability of durum wheat production in Tunisia

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The significant durum wheat productivity gains made in Tunisia over the last decades through both breeding for higher yield potential and stability and the implementation of improved agronomic practices have often been hampered by severe yield and/or income losses due to biotic constraints in the favorable northern part of the country. Several pathogens affect wheat in Tunisia, with different impacts on yield. Leaf rust, is present in most years, but comes generally late in the season and rarely at yield-limiting levels. However, the main productivity-threatening biotic constraint in Tunisia is, by far, Septoria Leaf Blotch (SLB). The prevailing strains seem to have developed a unique aggressiveness towards durum wheat. All commercial durum cultivars released previous to 2003 are highly susceptible to this pathogen, and yield losses in excess of 40% are not uncommon in years or environments with average to high rainfall. Extensive screening and crossing work done by the national program, lead to the release of Nasr in 2004, Maali in 2007 and Selim in 2009. All of these three cultivars have better disease resistance than the widely grown susceptible cultivar Karim. Under heavy epidemics of Septoria tritici, Nasr yields up to 42% higher than Karim. Results from seven years of replicated yield testing (2004-2010) show that Mâali has the highest yield potential of all previously released cultivars and a good level of resistance to SLB, though not as that of Nasr, under favorable conditions in Beja. Its wide adaptation, its good resistance to leaf rust and particularly to lodging put Mâali in the best position to take over significant acreage from Karim. More significant progress was achieved with the most recent release of Selim. This cultivar has shown the best level of resistance to SLB so far and a good resistance to leaf rust. The new generation of Septoria resistant (Nasr and Selim) or less-susceptible (Mâali) cultivars with outstanding agronomic performance is in a strong position to finally take-over from the landmark cultivars of the Tunisian durum “Green Revolution” and provides an opportunity for a more sustainable and hopefully less unstable, national durum production.

Identification of quantitative trait loci for resistance to Septoria tritici leaf blotch in durum wheat

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Septoria tritici blotch (STB), caused by the ascomycete *Mycosphaerella graminicola* (anamorph *Septoria tritici*) is an economically important foliar disease in the major wheat-growing areas of the world. In Tunisia, breeding for resistance is an effective way of controlling STB on durum wheat. In this study, resistance to STB in durum wheat was investigated in a population derived from a cross between the susceptible variety Karim and the newly released variety Salim. One hundred and forty-five single-seed descent derived F6 recombinant inbred lines (RILs) and parental lines were tested for resistance to Septoria under natural infection in field conditions during 2009 and 2010 seasons. Symptoms of STB were assessed by the Double digit 00-99 scoring method at early grain filling stage. A genetic map was constructed using the recombinant population genotyped with a total of 293 DArT markers and 22 SSR markers. Linkage analysis defined 29 linkage groups with a total map length of 795 cM. Most markers were mapped to the B-genome with length of 450 cM against 345 cM to the A-genome. A QTL for resistance to STB was identified and located on chromosome 3B. This QTL showed significant effect in 2009 and 2010 seasons with R^2 value of 23,47%. DArT markers linked to the QTL influencing resistance to STB have potential use in durum wheat breeding programs.

Identification of QTLs for resistance to Septoria tritici blotch in durum wheat through association mapping

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Septoria tritici blotch, caused by the fungus *Mycosphaerella graminicola* has been an important yield-limiting factor for durum wheat in North Africa and has recently spread around the Mediterranean Basin. The high genome plasticity of the pathogen and its specialization features (differential pathogenicity towards durum and bread wheat) hinder the identification and exploitation of resistance genes across diverse growing areas. The genetic variation of the response to *M. graminicola* and the chromosomal location of resistance factors were studied in a durum wheat panel of 183 accessions of diverse origin suitable for association mapping. Phenotypic evaluation of the panel was conducted in the field in four environments: Béja in Tunisia (2008, 2009 and 2010), Toluca in the Mexican highlands, Argelato and Ferrara in Italy, using local isolates. Additional phenotypic evaluation was conducted under controlled conditions at the seedling stage by inoculation with ten isolates from durum wheat collected from various Mediterranean countries. The panel was genotyped with 300 mapped SSR and some 900 durum wheat DArT markers. Significant marker-trait associations involving specific chromosomal regions have been detected using both field data (infection score at peak epidemics) and seedling assay data (percentages necrosis and pycnidia on primary leaves). Significant marker-septoria reaction associations related to photoperiod/vernalization genes (Italy) or height genes (Tunisia) were detected, but were not the only ones. A region on 1BL was found to include gene(s) related to resistance to both Tunisian and Italian field isolates. Most other associations were country specific. A preliminary analysis highlighted some chromosome regions consistently involved in resistance to Septoria tritici blotch in bread wheat, particularly in chromosomes 1BL, 2AL and 4AL that accounted for a sizeable portion of phenotypic variation among this panel. The detailed results will be presented and discussed.

Posters

Session 1

Pathogen Biology, Taxonomy and Evolution

Marker assisted selection for *Septoria tritici* resistance in some RIL wheat lines and Moroccan bread wheat genotypes

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Septoria leaf Bloch (STB) is a fungal leaf disease of wheat caused by *Septoria tritici* (*Mycosphaerella graminicola*). It is a big problem in temperate rainfall wheat (*Triticum aestivum)* Moroccans growing regions. Breeding for resistant cultivars is the best strategy to prevent the lost production and it's the most economical and efficient means of control. The aim of this work is to determine the molecular marker linked to Septoria tritici blotch disease in wheat morocean genotypes (*Triticum aestivum*). Some marker associated to Stb genes were used: Xgwm 493 linked to Stb2, Xgdm 132 linked to Stb3, Xgwm 111 linked to Stb4, Xgwm 44 linked to Stb5, Xgwm 146 linked to Stb8 and three SSR markers Xgwm 499, Xgwm 344 and Xgwm 285, are applied on a set of 19 of bread wheat Moroccan genotypes and the most resistant and susceptible F8 Recombinant –Inbred Lines (RILs) developed from a cross between Nasma susceptible Moroccan genotype to *Septoria tritici* and the synthetic hexaploid wheat " Syn8" developed by Dr. Mujeeb Kazi from CIMMYT and selected for resistance to *Septoria* for three years in Moroccan field conditions. For this propose, these markers are used in order to find out linkage between markers and resistance genes to *Septoria tritici* in Morocco. Amplification products specific for SSR markers linked with Stb5 gene and Xgwm 499, Xgwm 344 and Xgwm 285 marker are monomorphic for the resistant and susceptible lines in the field. So we do not have Stb5 gene in our resistance lines. The results obtained for Stb3 and Stb4 were inconclusive. L' amplification of Xgwm493primer linked to Stb2 loci generated 4 profiles of alleles different to those obtained by Tika et al. (2004). Profile 3, which include lines characterized by a resistance in the field. Applying this marker on Moroccan bread wheat genotypes, in profile 3 we found varieties Saada, Raja, and Amal, which are characterized by their tolerance. This leads us to conclude that these strains may harbor other Stb resistance genes to identify and characterize.

Posters

Session 2

Epidemiology and Population Biology

An experimental method to simultaneously characterize the effect of leaf temperature on the disease development on several leaves for a given temperature range

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Air temperature is commonly used to characterize the effect of temperature on foliar diseases. However, leaf temperature, different for each leaf within a canopy, differs from the air temperature measured within a canopy and ever more greatly if measured by a weather station. Such differences depend on the plants architecture and on climatic variables (wind, cloudiness, etc). Thus, the simultaneous measurement of the temperature of many infected leaves may be of great importance to study the actual impact of temperature on the different processes involved in the infectious cycle and establish temperature response curves, with possible consequences on the future disease forecasting. To generate and record a range of many infected leaf temperatures. We propose an experimental method that has been tested on *Mycosphaerella graminicola* for wheat plants grown in growth chamber and greenhouse. First, to easy replications and avoid artifacts from lighting, inoculated leaves were maintained horizontally. Second, the range of leaf temperature has been increased without impacting light incidence by using infrared heat lamps, positioned above some of the leaves. Third, the temperature of each leaf was measured with thin T-type thermocouples positioned under the inoculated area. Each thermocouple was connected to a datalogger, recording leaf temperature every 20 seconds, averaged per quarter hour. Due to the high number of leaves, several dataloggers were required. The contact of thermocouples with leaves was checked three times a week. Fourth, the measurement quality was assessed. The calibration of all thermocouples was carried out before and after the experiment. To avoid bias from using many dataloggers, the temperature of one brass block was continuously measured by each datalogger. This method has enabled the simultaneous measurement of 288 inoculated leaf temperatures. We obtained a wide range of mean leaf temperatures from 10 to 17°C in growth chamber and from 17 to 23°C in greenhouse, allowing the characterization of the relation between leaf temperature and the STB symptom development. This method enables several temperature treatments in a single growth compartment, well suited for climate change studies. It also could be applied to other foliar diseases. Its extrapolation to field conditions is under study.

Mating type distribution suggests sexual reproduction of *Mycosphaerella graminicola* in the French region Nord-Pas de Calais

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Mycosphaerella graminicola is nowadays the most frequent and the most economically damaging pathogen on wheat in France, especially in the Nord-Pas de Calais region, where the environmental conditions are suitable for the fungal epidemics. Four hundred and three monoconidial *M. graminicola* strains were obtained from fungal isolates sampled in 2009 from 17 fields covering this region. We aimed at examining mating type distribution in relationship with sexual reproduction. The sampling was carried out in most cases according to hierarchical sampling, by isolating 4 isolates from distinct plants in 6 m^2-field squares separated each other by 10 m, leading in average to 24 isolates per field. The mating type of each isolate was determined with multiplex PCR. Overall, 195 (48 %) out of the assessed isolates were MAT1-1 and 208 (52 %) were MAT1-2. Undergone χ^2 tests revealed that the two idiomorphs were equally distributed at the whole region scale ($P = 0.52 > 0.05$) as well as within each of the 17 sampled fields. At the field scale, both mating types were scored together in 83 (87 %) out of the 95 m^2-sampled squares, thereby putting forward the co-occurrence of the two mating types within the region. No relationship was found between mating types and either infected wheat cultivars, disease pressure level or crop systems. The even distribution of the two mating types reported here strongly suggests a large potential for sexual reproduction of *M. graminicola* in Nord-Pas de Calais and confers suitable conditions for both teleomorph formation and high genetic diversity that might lead to local adaptations to environmental pressure.

The intraspecific structure of populations of *Septoria tritici* (teleomorph *Mycosphaerella graminicola*) on wheat in different regions of Russia

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Septoria tritici Rob. et Desm. is one of the most widespread Septoria agents on wheat in Russia. By frequency of occurrence this pathogen dominates in south and central areas of country (North-Caucasian, Central-Chernozem and Central regions), where its share in septorioses complex is 60-80%. Moreover, it occurrs in Volga and Siberia regions. *S. tritici* isolates are characterized by variability of cultural-morphological features on nutrient medium. The isolates are separated into three main groups of colonies (yeast-like, mixed and mycelial) and 10 morphotypes. The ratio of morphotypes varies in different regions of RF. The virulence of 140 single-spore *S. tritici* isolates from three regional populations (North-Caucasian, Central-Chernozem and Central) was determined on the cultivars with known resistance genes: Bulgaria 88 (*Stb*1), Oasis (*Stb*1), Veranopolis (*Stb*2), Israel (*Stb*3), Tadinia (*Stb*4), CS/Synthetic 70 (*Stb*5), Estanzuela Federal (*Stb*7). The virulence was studied at seedling stage by the percentage of infected leaf area and by fungus sporulation *in vivo*. The isolates had different virulence spectrum. In whole, the number of virulent isolates to cultivars with *Stb*1, *Stb*5 and *Stb*7 genes was greater, than to cultivars with *Stb2*, *Stb3* and *Stb4* genes (25,6-61,8% and 1,8-34,0%, respectively). None of *Stb* genes was effective to all *S. tritici* isolates. The effectiveness degree of some *Stb* genes to different *S. tritici* populations was unequal.

Posters

Session 3

Pathogen Genetics and Genomics

Genetic diversity of the wheat pathogen *Mycosphaerella graminicola* in Morocco

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Septoria tritici blotch caused by the fungal pathogen *Mycosphaerella graminicola* is currently one of the most frequently reported diseases on both bread wheat and durum wheat in Morocco. A total of 96 single-conidial isolates of the fungus were sampled in 2010 during the growing season from 6 Moroccan wheat producing regions (Tadla-Azilal, Doukkala-Abda, Chaouia, Fes-Boulmane, Gharb-Chrarda and Meknes-Tafilalet) in order to examine the genetic diversity of the Moroccan population of *M. graminicola*. The genotyping of the isolates was performed using 8 microsatellite markers: ST1A4, ST2C10, ST1D7, ST1E3, ST2E4, ST1E7, ST1G7 and AC0007. The highest number of alleles was obtained for ST1D7 showing 8 distinct alleles, while the lowest number of alleles was found with ST2E4 for which only 3 alleles were observed. In average, 5.25 alleles were obtained per marker. Statistical analyses revealed that the assessed population consists of 60 % of unique haplotypes, corresponding to a clonal fraction of 40 %. Identical clones were mainly collected from a same lesion, a same leaf or a same region. Further analyses using POPEGENE and STRUCTURE software will be undertaken in order to determine additional genetic parameters allowing a better characterization of both the genic and genotypic diversity and the population structure of *M. graminicola* in Morocco.

Substitutions in the *CYP51* gene and triazole performance against *Mycosphaerella graminicola*, the cause of leaf blotch of wheat

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Septoria leaf blotch is the most economically important foliar disease of wheat in UK, France and many other European countries. Because of the lack of the high resistance wheat cultivars, the main method to control the disease is by using fungicides. Sterol 14α-demethylation inhibitors (DMI) which belongs to sterol biosynthesis inhibitors (SBI), also called triazoles, have been played an important role against *M. graminicola* for last few decades. Over the past of 10 years, many reports indicated the field performance decline of DMIs. Changes in Cyp51gene, encoding the sterol 14α-demethylase, is one of the mechanisms causing reduction in the sensitivity of *M. graminicola* isolates. Molecular analysis of Cyp51 gene of 18 *M. graminicola* isolates has revealed the existence of 13 alterations or deletions. The bioassay results has revealed great differences in fungicide resistance levels among genotypes and therefore, the existence of 6 phenotypes found displaying reduced sensitivity to DMIs. Combinations of the changes I381V, Y459S and/or A379G caused significant reduction in DMI performance *in planta*. Furthermore, the high frequency of the change I381V caused high resistance to tebuconazole and other DMIs, but not prochloraz. Likewise, S50L combined with Y461H or Y136F, also caused high resistance to tebuconazole and lower resistance to prochloraz and other DMIs. However, the mutations V24D, S50L, S51T, V136A and Y137F, alone or in combination, resulted in only a small shift in sensitivity to triazoles.

Role of MAP kinases pathways in the infection process of the wheat pathogen *Mycosphaerella graminicola*

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Mitogen-activated protein kinases (MAPKs), are essential components of evolutionary conserved signaling pathways in eukaryotic cells. *Mycosphaerella graminicola* a worldwide pathogen of wheat causing Septoria tritici leaf blotch (STB), has three MAPK pathways depending respectively on kinase *MgFus3, MgHog1* and *MgStl2*. These three signaling pathways are involved at different stages of the infection process. To decipher the infection related mechanisms controlled by these signaling pathways, we are developing a combination of molecular approaches. Comparative transcriptomics will be performed using null mutants from these three genes (*MgFus3*, *MgHog1*, *MgStl2*) and wild type strains with genome wide DNA microarrays grown under conditions corresponding to either an active or an inactive pathway. Additional transcriptomic analyses will rely on conditional MAPK mutants to better control the activation/inactivation status of these pathways. Genes whose expression (induction, repression) require an active MAPK will be further studied using reverse genetics and biochemistry (phosphorylation status, protein-DNA interaction).

Posters

Session 4

Host Pathogens Interactions

Parameters of partial resistance on wheat cultivars to *Stagonospora nodorum* and *Septoria tritici*

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Dynamics of Septoria development on more than 2500 wheat samples from a world germlasm was studied in an infectious nursery of All Russian Research Institute of Phytopathology. The area under the disease progress curve (AUDPC) and resistance index (IR) were calculated. For studying parameters of partial resistance (the latent period, the size of infectious spots) wheat cultivars with high and average indexes of resistance to Septoria were selected. The wheat samples were conditionally divided into 4 groups by the duration of latent period and size of infection spots: 1 - Samples with small infection spots and long latent period; 2 - Samples with small infection spots and short latent period; 3 - Samples with large infection spots and long latent period; 4 - Samples with large infection spots and short latent period. Wheat cultivars of the first group characterized high level of partial resistance in the infectious nursery are the most important for breeding (Anderson, PI 278222 (the USA), RAC 610, RAC 569, K-20 (SAR) and SWS"A" №80 (Sweden) infected *S. nodorum* and cultivar Galina infected *S. tritici*). Wheat cultivars Warigal*Dagger, Cltr 278222, Cltr 604225 and Enita to *S. nodorum* and Piamontes to *S. tritici* concerned to the second group. Cultivars Voronezhskaya 10, Lyra 98, Legenda, Owens to *S. nodorum* and cultivars Azteca, Alondra to *S. tritici* were included into the third group. The duration of the latent period for them is more than for control cultivar Priokskaya 1.

Exploring quantitative relationships between mycelial growth and visual symptoms of *Septoria tritici*

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Quantitative PCR (q-PCR) allows for a measurement of mycelial growth *in planta*. This technology has been extensively used for the study of Septoria tritici blotch (STB), arrival of primary inoculums, disease resistance and escape to its long-term dynamics. The results reported here are the first to attempt to quantitatively link mycelial growth as measured by q-PCR to the evolution of necrotic symptoms on leaves. The results from three field experiments and a previously published data set show that, for a given leaf layer, mycelial quantity and necrotic area are strongly related. Mycelial quantity at a given time can be used to predict the thermal duration until onset of necrotic symptoms, approached by time to 10% necrotic symptoms obtained by fitting a gompertz curve to necrotic symptoms. For a given experiment, the ratio of necrotic area per unit of mycelium (log-transformed qPCR value), plotted over thermal time from onset of symptom development, follows an exponential or quadratic pattern. This empirical evidence at the field level calls for a mechanistic approach to modeling the quantitative link between mycelial growth and symptom development, which could serve as a general framework for understanding STB resistance.

Posters

Session 5

Cultural Measures and Disease Management

Co-occurrence of *Phaeosphaeria nodorum* and *Mycosphaerella graminicola* in winter wheat in Lithuania

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The co-occurrence of *M. graminicola* and *P. nodorum* on winter wheat crops was observed in Central Lithuania over the period 2007-2010. The first symptoms of both *M. graminicola* and *P. nodorum* on the upper three leaves were detected at booting stage, except for 2010, when unusually early and severe *M. graminicola* infection started from the beginning of stem elongation. Irrespective of the differences in rainfall amount and temperatures between the experimental years, a clear predominance of *M. graminicola* over *P. nodorum* was observed annually. *P. nodorum* infection on ears varied depending on the amount of rainfall during the ripening stage. In a particularly wet season of 2010, *P. nodorum* infection on ears was especially severe on short-strawed, later-maturing cv. Kovas compared to longer-strawed, earlier-maturing cv. Mulan. A relationship was established between disease infection level on ears and harvested grain infestation by *P. nodorum* on SNAW (*S. nodorum* agar for wheat) - 24% of grain of cv. Kovas and 11 % of Mulan were infected. Fungicide treatment at booting provided an effective control of both *M. graminicola* and *P. nodorum* infection on leaves, but gave only slight efficacy against *P. nodorum* infection on grain on both cultivars tested. Wheat seed infection by *P. nodorum* is an important source of primary inoculum for foliar epidemics, which has been evidenced in 2011 by the appearance of the first symptoms of disease on upper leaves at early booting in fallow - drilled winter wheat fields following the severe *P. nodorum* infection in 2010.

Leaf spotting diseases in winter and spring wheat and resistance to fungicides in *Mycosphaerella graminicola*

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In Lithuania, the most destructive pathogens in wheat production are *Mycosphaerella graminicola* (*Septoria tritici*), *Phaeosphaeria nodorum* (*Stagonospora nodorum*) and *Pyrenophora tritici-repentis* (*Dreschslera tritici-repentis*) As a new disease, tan spot was recorded in Lithuania in 1997 in commercial fields and is the most prevalent leaf spotting disease, especially under continuous wheat cropping. Earlier studies showed that *P. nodorum* prevailed over *M. graminicola;* however, in recent years the domination of Septoria leaf blotch has been noted. Outbreaks of this disease were recorded in 2007 and 2010 when the severity of Septoria leaf blotch reached 30-75% in the untreated plots at milk growth stage. Septoria and Stagonospora leaf blotches and tan spot significantly affect grain yield in both winter and spring wheat crops. Research on leaf spotting diseases in winter and spring wheat indicated that a disease severity ranging between 1-5% was responsible for 5.5% grain yield reduction compared to healthy plants. Fungicides are important tools for managing leaf spotting diseases in wheat. However, pathogens' resistance to fungicides has become one of the most critical problems recently. The first surveys on *M. graminicola* resistance to the QoI group fungicides were made by pesticide companies in 2004 in Lithuania, and the first resistance isolates were found in 2005 and reported by FRAC. Monitoring of resistance has been carried out at our institute since 2008. The sensitivity of *M. graminicola* to QoI (azoxystrobin and pyraclostrobin) and DMI (epoxiconazole, cyproconazole and prothioconazole) fungicides is being tested in vitro using microtiter plate test. The *M. graminicola* resistance to QoI and DMI fungicides is being assessed also as part of resistance monitoring in Nordic–Baltic countries according to NORBARAG (Nordic-Baltic Resistance Action Group) activity. Increasing resistance to QoI and emerging resistance to DMI fungicides in *M. graminicola* obligate researches, advisers and cereal growers to review the existing control strategies. Recommendations for cereal disease control as given by NORBARAG's fungicide subgroup with respect to minimizing the risk of fungicide resistance are forwarded to Lithuanian cereal growers. Strobilurins are to be applied preferably once per season, DMI fungicides should be combined with the fungicides differing in the mode of action.

Effect of wheat debris as source of primary inoculum on the early stages of Septoria leaf blotch epidemics

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Management strategies of Septoria leaf blotch could be improved by a better characterization of the onset and early stages of epidemics. The inoculum build-up and overseasoning potentially involve various fungal structures (ascospores, pycnidiospores, mycelium) and plant material (wheat seeds, stubble and debris; wheat volunteers; other grasses). Among the mechanisms reportedly involved in disease establishment and year-to-year disease transmission, infection by ascospores, wind-dispersed from either distant or local infected wheat debris, is considered as the most significant. Nevertheless, infection by pycnidiospores, splash-dispersed either from neighbouring wheat debris or from senescent basal leaves, has also been inferred from indirect evidence. The contribution of wheat debris to the early **s**tages of Septoria leaf blotch epidemics and the relative importance of different types of inoculum to the establishment of *Mycosphaerella graminicola* were assessed in a three-year, field experiment conducted in the Paris Basin, a main wheat producing area in France. Three options of debris management (chopped debris, removed debris followed by tillage, tillage in absence of debris) were tested, which had a strong effect, although transient, on the early epidemic dynamic: the more debris present on the soil surface, the more severe initial disease was. The relative production of pycnidiospores and ascospores was measured on the chopped debris. Autumn conditions, characterized by moderate temperature with alternating wet and dry periods, were favorable for the production of ascospores and pycnidiospores, which concomitantly peaked in October-November; thus both types of spores can be involved as primary inoculum in North-West European conditions. During the winter period, the decrease in primary inoculum delayed disease progress; later on, disease progress resumed because of the input of inoculum from remote origin (ascospores). Management of wheat debris at the plot scale cannot be expected to decrease final disease severity and yield loss, although it can reduce the local amount of primary inoculum. Such a decrease, however, is likely to be obtained by limiting inoculum on a larger scale, considering that local inoculum sources in a given plot will also act as inoculum sources for distant plots.

Combined treatments to reduce *Septoria tritici* in wheat and their impact on crop yield and its components

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Field experiments were conducted to analyze the effect of *Trichoderma harzianum* as natural biofungicide of *Septoria tritici* in wheat. The effect of two biocontrol strains of *T. harzianum* alone or in combination with a fungicide were evaluated on the severity of leaf blotch of wheat (LBW) with different application techniques at three growth stages (seedling, tillering, heading).The impact of these treatments on spikes number/ m^2, grains per spike, thousand kernel weight (g) and grain yield (kg ha^{-1}) were studied. Plants of Buck 55CL2 variety received different treatments: control plants inoculaed with *S. tritici*; plants from coated seeds with the strains (Th5cc, Th118) of *T. harzianum* and inoculated with the pathogen; plants from coated seeds and spray application on leaves of conidial suspension of *T. harzianum* alone or in combination with an aerial fungicide (Amistar Xtra) sprayed 7 days before the inoculation with the pathogen. With respect to severity, at seedling and tillering, only necrotic tissue development was assessed by the low incidence of pycnidia. At heading, only the pycnidial coverage percentage was assessed because of high incidences of necrosis in all treatments. With only coated seed treatment the reduction in the pycndial coverage percentage was maintained until heading stage ($p \geq 0.05$). Lower values of necrosis were obtained when seeds were coated with the antagonist and seedlings were also sprayed with half-doses of fungicide ($p \geq 0.05$).When applying the single antagonist the use of the strain Th5cc is recommended with 3 applications(coated seed and two spray applications at tillering and heading) that decreas the pycnidial coverage until heading ($p \geq 0.05$). The analysis of the yield components showed that spikes /m^2 and thousand kernel weights were altered by the combined treatment ($p \geq 0.05$).

Trichoderma harzianum as inductor of a biochemical defense responses against *Septoria tritici*

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The use of antagonists, single or combined with fungicides, is proposed an important tool for the leaf blotch of wheat management. The purpose of this study was to demonstrate the induction of a biochemical response when *T. harzianum* strains actived an antifungal action against *Septoria tritici* of wheat at three growth stages The experiment was conducted in an experimental field during 2009 and 2010. A block design with 18 treatments and 3 replications was used. The treatments were: 2 strains of *T. harzianum* (Th5cc and Th118) that were applied as a seed coating and as a leaf spray at two growth stages (tillering) and (heading); healthy control plants(T) without the pathogen and the antagonists and control plants (Ti) inoculated with *S. tritici*. After 21 days of *S. tritici* inoculation, the extent of the leaf necrosis area and the pycnidial coverage percentage were estimated for each growth stage on Buck 55CL2 wheat cultivar. In addition, the leaf apoplast antifungal proteolytic activity was measured in plants 22 days after sowing. The proteolytic activity of the intercellular wheat fluid (IWF) and the endogenous germin-like protease inhibitors (GPI) were demonstrated throughout the growth stages till maturity. The literature shows that the antifungal action decreased in plants that were only inoculated with *S. tritici* but increased in those grown from seeds coated with the *T. harzianum* (Th5cc) strain. This increase stimulated defenseses to the wheat cultivar. For the present experiment the proteolytic activity of the (IWF) decreased on (Ti.) at tillering and heading. The proteolytic activity was similar to the healthy control when plants coming from coated seeds were sprayed with *T. harzianum* in both mentioned stages. The proteolytic activity was regulated by the GPI activity. At heading, the GPI was almost undetectable in the IWF of the flag leaf. Even so, its inhibitory activity was critical in the regulation of proteases. It is a first time that we have seen that *T. harzianum* stimulates a biochemical defense response in plants inoculated with *S. tritici* in all growth stages.

Mapping distribution of Septoria allowed targeting management options of the disease

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Durum wheat (*Triticum turgidum* L var. *durum),* is the most important cereal crop in Tunisia, it occupies about 2/3 of cereal grown area. The average yield of durum wheat ranges from 1.5 to 3.0 t/ha under rainfed conditions and about 4-6t/ha under irrigated conditions. The low yield is mainly due to extended drought conditions and to fungal diseases that become an important limiting factor during rainy seasons. Septoria Leaf Bloch *(Mycospharella graminicola)* is the major leaf disease which cause significant yield loss that varies between 5% and 35% and over than 60% during epidemic conditions. In 2003, 2004, 2005 and 2006 growing seasons, commercially grown durum wheat fields in Northern Tunisia were surveyed for Septoria leaf blotch at the tellering and stem elongation growth stages. The prospected areas represents the major durum wheat areas in the country where the annual rainfall is relatively high (>500 mm) in the sub-humid areas to relatively low (<500 mm) in the semi-arid areas. Onset of septoria disease was recorded in prospected fields during 2002 and 2003; and disease incidence was estimated during 2004 and 2005 crop seasons. Development of Septoria leaf blotch disease varied from year to year within and between regions. Five different regions were identified on the basis of disease development. Septoria diseases hot spots identified in the sub humid and semiarid areas were characterized by early infection establishments at the beginning of winter season (mid- December and mid- January respectively). Epidemic disease development occurs later in the season (mid-March). Disease infections in the medium spot area were detected towards the end of winter (mid- February) and an epidemic disease development was concurrently encountered under conductive climatic conditions. Disease infection was rarely observed in the semi-arid regions. A GIS disease map was developed and has allowed detection of risk areas and hence better management of disease control program. Integrated disease management options were then proposed for each of the Septoria hot spot areas.

Evolution of sensitivity to azoxystrobin and triazoles in Belgian *Mycosphaerella graminicola* populations and effects of fungicide treatments under field conditions

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Since the early 2000s, the evolution of sensitivity to azoxystrobin and azoles in Belgian populations of *Mycosphaerella graminicola* has been determined in microtiter assays. The rapid and widespread increase in resistance to azoxystrobin was observed over 4 years, with the percentage of resistant strains being less than 4% in 2002 but exceeding 90% after 2007. Tests carried out on 1,569 strains from 2002 to 2009 showed a reduction in sensitivity to epoxiconazole: the median EC50 value increased from 0.04-0.08 µg/ml for strains collected in 2002 to 0.16-0.32 µg/ml for strains collected in 2006. A further increase in resistance to epoxiconazole was not apparent in strains collected from 2006 to 2009. The tests for sensitivity to propiconazole, performed on 1,407 strains from 2000 to 2009, revealed an increase in the median EC50 value from 0.04-0.08 µg/ml in 2002 to 0.16-0.32 µg/ml in 2008 and 2009. Nevertheless, an analysis of the sensitivity of 689 strains to tebuconazole revealed an increase of the median EC50 value from 0.32-0.64 µg/ml in 2007 to 0.64-1.28 µg/ml in 2008 and 2009 A comparison of the levels of sensitivity to the three triazoles showed that resistance to tebuconazole was not correlated with resistance to propiconazole or epoxiconazole. This could be linked to the various mutations in the target-encoding CYP51 gene in *M. graminicola* associated with changes in sensitivity. The sequencing of parts of this gene for 33 strains collected in Belgium from 1993 to 2007 reveals the existence of more than 20 variants. The effects of seven fungicide treatments in the field on the levels of resistance of the strains to epoxiconazole, propiconazole and tebuconazole differed from one treatment to another. A large distribution of EC50 values of the strains was consistently observed. The variable effects of the treatments with triazoles and/or carboxamide (boscalid, penthiopyrad) highlighted the important of using different fungicide families to reduce the selective pressure on *M. graminicola* populations.

Effect of wheat canopy architecture and rain characteristics in splash of *Mycosphaerella graminicola* pycnidiospores

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Wheat architecture can modify the progression of Septoria leaf blotch epidemics within the canopy. Our goal was to quantify experimentally splash dispersal in varied plant architectures and for different types of rain. Disease progression due to secondary contaminations is mainly caused by rain splash process which enables upward vertical and lateral pycnidiospore dispersal. We investigated in controlled conditions both the effect of plant architecture and rain characteristics on the dispersal of *Mycosphaerella graminicola* conidia. The experiment was performed using a rainfall simulator in wheat canopy microcosms of 1 m^2, grown in a regulated temperature greenhouse. Two quasi-isogenic lines of Mercia wheat (differing only by one dwarf gene: RHT) were selected to avoid varietal factor that could modify disease progression (such as varietal resistance). Artificial inoculum source was chosen to avoid difference in sporulation due for example to the modification of micro-climate. The inoculum was a rectangular 100 cm x 5 cm saucer full of conidia suspension (10^6 conidia.mL^{-1}) localized in the center of each canopy. *M. graminicola* isolate was collected in field from typical Septoria leaf blotch lesion on Mercia wheat. Each canopy was exposed to two different rainfall events with two repetitions: a classical stratiform rain with raindrop diameter distribution centered on 2.5 mm, and a heavy thunderstorm rain with raindrop diameter distribution centered on 3.0 mm. The most relevant architecture components (internodes, wheat height, leaf size) were assessed just before each rainfall event. Thereafter, canopies were incubated in a mist chamber for 4 days, and kept 21 days in a greenhouse at 20°C during the symptomless phase (latency period). Disease symptoms (diseased and green surfaces) resulting from rain splash and leaf positions in the canopy were precisely assessed. Results showed here the specific effect of architecture and of type of rain in the dispersal of conidia.

Posters

Session 6

Tan spot and other Septoria Like Diseases

Effectiveness of fungicides against Stagonospora nodorum blotch and tan spot of winter wheat in Georgia

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Georgian biological, geographical and climatic conditions have given rise to a great diversity of plants. Most important is that Georgia is one of the main genetic centers of origin of wheat, according to historical, archeological and biological finding. Leaf spot of wheat (*Triticum aestivum L.*) in Georgia consists of a group of diseases involving Stagonospora nodorum blotch (*Phaeosphaeria nodorum* (E.Muller) Hedjarroude), Tan spot (*Pyrenophora tritici-repentis* (Died.) Drechs.), and Septoria tritici blotch (*Mycosphaerella graminicola* (Fuckl) J. Shrot). A complex of these diseases occurs in nature hence managing leaf spots is difficult. The goal of our study was the estimation of harm of wheat Stagonospora nodorum blotch and tan spot and efficiency of fungicides against these diseases. The work was conducted in Infection Nursery at Institute land (Kobuleti) according international methods. Inoculation of the six of the most cultivated in Georgia varieties (Vardzia, Bezostaya 1, Krasnodarskaya 99, Russa, Pobeda 50 and Copper) was made with mix of strains, which were collected in the different geographical zones of Georgia. System fungicides – Tilt 250, Alto Super and Bayleton were used for experiments. Data analysis has shown that both diseases are quite harmful for wheat in Georgia. The loss of yield varies within 15 – 49 % (depended on varieties). The most effective were fungicides Tilt 250 and Alto Super in comparison with Bayleton. These preparations decreased the defeat index by 31 – 60 % and increased harvesting by 14 – 25 % (depended on varieties).

Broad based genetic resistance to tan spot of wheat in CIMMYT germplasm

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Tan spot, incited by the ascomycete fungus *Pyrenophora tritici-repentis* (Died.) Drechs, is a major foliar disease of wheat. *Pyrenophora tritici-repentis* induces two distinct symptoms on susceptible wheat cultivars, tan necrosis and extensive chlorosis. Tan spot causes on average yield losses of 5-10%, however under conditions favourable for disease development losses can be higher than 50%. Significant reduction in grain quality is manifested by tan spot due to grain shriveling, red smudge and black point. Conservation agriculture in combination with wheat monoculture involving cultivation of susceptible cultivars has resulted in frequent onset of tan spot epidemics worldwide. Increasing temperatures and drought due to climate change could result in a higher degree of leaf senescence which is likely to further increase tan spot severity. Development of resistant cultivars, in conjunction with crop rotation, will provide an effective, economical, and environmentally safe means of controlling tan spot. CIMMYT, Mexico in collaboration with SLU, Sweden has initiated major efforts to mitigate the threat of tan spot. Efforts include large scale screening of wheat germplasm under traditional seedling and hydroponic tests and association analysis for identification of genomic regions contributing to resistance. Screening experiments reveal that CIMMYT's elite bread wheat germplasm has a high level of resistance to tan spot caused by *P. tritici-repentis* race 1. Resistant breeding lines have diverse genetic make-up and the resistance is likely broad based. Association mapping studies done with CIMMYT germplasm reconfirmed that, in addition to the previously identified genomic regions contributing to tan spot resistance, CIMMYT germplasm has novel genomic regions on the long arm of chromosomes 6A and 7B for tan spot resistance. Efforts are being made to incorporate this broad genetic resistance into modern wheat cultivars.

Development of tan spot of wheat in Georgia

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Tan spot, sometimes called yellow leaf spot, is an economically important disease in all the major wheat growing areas worldwide. Georgia is not exception, as climate and wheat growing conditions favorable for wheat production also favor tan spot development here. Mainly, winter wheat varieties are growing in Georgia. The tan spot fungus survives and produces on infected wheat straw and spreads by ascospores in the spring, and during whole vegetation season - by asexual spores, called conidia. Disease development favored by period of overcast, high humidity with often rain and warm temperatures. The first symptoms of tan spot were mainly observed at Z37 (flag leaf just visible) growth stage of crop. High level of disease was indicated at late milk-early dough ripe stage of wheat. Accordance with results of 10 years surveys tan spot has been recorded in all sowing areas of Georgia and on all released wheat varieties. Survey locations generally were not different by pathogen incidence. 548 pure cultures of fungi from different climatic zones of Georgia have been isolated and cultivated as pure culture. Cultural and morphological peculiarities of fungi were investigated on three media: PDA (Potato Dextrose Agar), CPA(Carrot Potato Agar), V4 (Tomato Potato Carrot Beet Agar). Especial abundant sporulation was recorded on CPA media. Two types of fungi has been revealed: Colony – dirty gray, velvet, fast-growing aerial mycelium with sporulation on the colony center and black substrate; Colony -dark-grey or blackish, velvet, moderate growing, aerial mycelium with black substrate and abundant sporulation on whole colony surface. Breeding material of different origin was evaluated under natural infection of *Pyrenophora tritici-repentis* in four climatic zones of Georgia during 2007-2010 growing seasons. The majority of the tested entries were susceptible to tan spot (incidence 30-100%, severity 5-60%). Seven endemic species and twelve variations were evaluated under artificial infection of Georgian population of *Pyrenophora tritici-repentis.* Resistance to tan spot was detected in 26% of the tested entries.

Posters

Session 7

Host Genetics and Resistance Breeding

Screening wheat germplasm for Septoria resistance in Ethiopia

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Wheat is the major cereal grown on 1.8 million hectares of land every year in Ethiopia. National average wheat yield is below 2 t ha^{-1}. Septoria is one of the major diseases limiting wheat yield. The two prevalent species in the country are: *Mycosphaerella graminicola* (Fuckel) Schort (*Septoria tritici*) that infects the leaves and *Stagonospora nodorum* (*Septoria nodorum)* that infects both the leaves and the glumes. The use of resistant variety is the best economically feasible and environmentally safe method of control. The objective of this screening was to identify Septoria resistant high yielding wheat lines. Numerous wheat germplasm were evaluated at several locations in the country. Holetta, Adet, Asasa, and Bekoji were identified as "hot spots" for Septoria. Septoria was scored using double digit 00-99 scale where the first digit is the relative progress of the disease in relation to plant height while the second score is the severity of the disease. Septoria pressure was higher on research stations than on farmer's field. Most wheat germplasm evaluated were found to be susceptible with early necrosis and leaf senescing which have resulted in lower yields. Very few promising lines showed moderate resistance to Septoria. These lines were either tall or late. Lines differed in severity than in relative disease progress. Septoria prevalence has increased in recent years because of the mono-cropping wheat after wheat in the major wheat belts of the country. Management practices such as crop rotation and removal of stubble were found to reduce the intensity. Some cultural practices such as seeding rates, seeding methods and fertilizer rates were found to have less effect on disease intensity. Septoria will continue to be a threat until resistant genes to prevalent isolates are identified and used in the breeding program. Thus breeding for Septoria resistance is a major goal in developing high yielding wheat varieties in Ethiopia.

Agropyron elongatum: a novel source of resistance to *Mycosphaerella graminicola*

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Septoria tritici blotch (STB) caused by *Mycosphaerella graminicola* is one of the most destructive foliar diseases in wheat. Single resistance genes have been among close wheat relatives, inculding *TmStb1* from *Triticum monococcum*. *Agropyron elongatum* (syn *Thinopyrum ponticum*), a tertiary gene pool for potentially favorable traits, has been extensively used in wheat improvement. Complete resistance to STB under field conditions was observed in a wheat-*Agropyron* amphiploid of unclear origin (designed PSR3628, from the so-called Sandoz material). Cytogenetic studies using *in situ* hybridization coupled with pathological tests were initiated to identify chromosome(s) carrying the resistance gene. The amphiploid has 56 chromosomes that includes 18 chromosomes of *Agropyron* and 38 chromosomes of wheat. Based on *in situ* probing, there are 14 chromosomes of *A. elongatum* (genome E) and four of some other related genome. As of now, it is still unknown which wheat chromosomes are missing. PSR3628 was crossed and backcrossed twice to *T. aestivum* cv. Pavon 76, the progenies were screened by the *in situ* probing, plants with between one and five labeled chromosomes were selected and grown. Their progenies were tested for resistance to STB. Plants with resistance to the pathogen were grown to maturity and their progenies were again screened by in situ probing with the same probe. Those with *Agropyron* chromosomes were grown, backcrossed again and their progenies were screened for resistance. The process was repeated three times. In all instances resistance to STB correlated with the presence of a single chromosome of the E genome, here labeled D, or its long arm.

Fine mapping of a QTL for resistance to Stagonospora nodorum glume blotch in wheat

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Resistance to Stagonospora nodorum glume blotch is quantitative. Three Quantitave Trait Loci have been previously identified in wheat genome by our group. The major QTL explains 31.2 percent of the phenotypic variation and locates on the short arm of chromosome 3B. In order to isolate this QTL by map-based cloning we developed a high-resolution mapping population consisting of Near Isogenic Lines derived from a cross of resistant Swiss winter wheat cultivar Arina and susceptible Swiss winter wheat cultivar Forno. Parental cultivars demonstrated contrasting phenotypes in the field infection tests. Construction of the genetic map benefited from the physical map of 3B chromosome as well as from sequence analysis of fragments available for the target region. Phenotyping of the fine mapping population was conducted using artificial infection in three field sites in Switzerland.

Selection of initial wheat material for resistance to *Stagonospora nodorum* and *Septoria tritici* from different genetic collections

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At the present time septoriosis has leading position among the big number of harmful fungi diseases of crops. Everywhere epidemics of the disease occur 4-5 times in every 10 years and crop losses can reach 40-50%. The species of *Septoria* are the most widespread in Russia. In the ARRIP infectious nurseries (the Central region of the Russian Federation) the long-term researches for resistance of wheat cultivars from different genetic collections to *St.nodorum* and *S. tritici* were carried out: ARIPI collection - 570 samples, Germplasm Resources Information Network (GRIN) - 1815 samples, CIMMYT collection - 132 samples. A mix of pathogenic races as an infectious background was used for field test. Wheat cultivars from all tested collections were characterized by leaf susceptibility to *S. nodorum*. Five leaf resistant to *S. tritici* cultivars from GRIN collection were selected. Fifty-five ear resistant cultivars to *S. nodorum* were revealed in GRIN collection including accessions from Brazil, the USA, Bulgaria and Mexico. Six resistant wheat cultivars from ARIPI collection were resistant to *S. nodorum* including what accessions from Brazil and China. Cvs. Anderson, Long 94-4081, Cooperation Calquin, Prointa Superior, Buck Arrayan represent the greatest interest resistant not only to septoriosis but also to leaf rust and powdery mildew.

Occurrence of Septoria leaf blotch in Algeria and assessment of wheat resistance

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Septoria tritici (*Mycosphaerella graminicola)* and *Stagonospora nodorum* (*Phaeosphaeria nodorum)* causal agents of Septoria leaf blotch of wheat occur throughout the major wheat growing regions in Algeria. The Septoria diseases are the source of major damage on susceptible varieties of bread and durum wheat. Surveys have been performed in Algeria during the last decade, they covered wheat growing areas of eastern and central regions, mainly in coastal areas (Annaba, Skikda, Tizi Ouzou, Boumerdes, Algiers) and sub-littoral (Guelma, Souk Ahras, Constantine, Sétif, Mila, Bouira). The disease development was always observed except during 2000-01 and 2001-02 growing seasons because of the excessive drought conditions. The surveys showed that Septoria diseases are more prevalent in coastal areas and sub-littoral where risk is very high. The surveys also revealed the presence of both Septoria species (*M. graminicola* and *P. nodorum*), but M. *graminicola* was more prevalent. Algerian National Wheat improvement Program in collaboration with ICARDA conducts routine screening for resistance to Septoria diseases since 2005. A collection of 150 durum wheat and 100 bread wheat genotypes were tested under natural conditions at two Septoria hot spots representing high and low rainfall areas, where the foliar diseases are normally expressed each year under field conditions. Wheat lines that carry resistance to Septoria leaf blotch were identified. Subsequently, resistant lines were tested under controlled condition. Introduced and local germplasm with resistance to Septoria were identified.

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