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**Durability assessment of oilseed rape resistance to
blackleg: Nine years of recurrent selection of
Leptosphaeria maculans populations in field experiment**

Hortense Brun, Isabelle Fudal-Grolier, Magali Ermel, Anne-Laure Besnard,
Lydia Bousset, Bruno Marquer, Frederique Eber, Michel Renard, Anne-Marie
Chèvre, Régine Delourme

► **To cite this version:**

Hortense Brun, Isabelle Fudal-Grolier, Magali Ermel, Anne-Laure Besnard, Lydia Bousset, et al.. Durability assessment of oilseed rape resistance to blackleg: Nine years of recurrent selection of Leptosphaeria maculans populations in field experiment. Plant resistance sustainability 2012. International conference, Oct 2012, la Colle sur Loup, France. hal-01190683

HAL Id: hal-01190683

<https://hal.science/hal-01190683v1>

Submitted on 3 Jun 2020

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PLANT RESISTANCE SUSTAINABILITY

International Conference _____ 2012



La Colle-Sur-Loup (France)
October 16th-19th, 2012



Scientific Programme and Abstracts

Sessions

Session 1: Impact of plant disease resistance on the structure and evolution of pathogen populations

Session 3: From plant-pathogen molecular interactions to the durability of resistance

Session 2: Sustainable and integrated breeding and deployment of genetic resistance

Session 4: Socio-economic issues related to the use of resistant varieties and their deployment in agro-systems

Invited Speakers

Philippe Baret, Université Catholique de Louvain, Belgium - **James Brown**, John Innes Centre, England - **Marion Desquilbet**, INRA, France - **Sylvain Gandon**, CNRS, France - **Benoit Moury**, INRA, France - **Chris Mundt**, Oregon State University, USA - **Laura Rose**, Heinrich-Heine University, Germany - **Walter Rossing**, Wageningen University, The Netherlands - **Peter Thrall**, CSIRO Plant Industry, Australia

Organised by the Institut National de la Recherche Agronomique (INRA)
Metaprogramme on Sustainable Management of Crop Health (SMaCH)



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Conference Presentation

A main challenge currently facing agricultural research is to find efficient and sustainable ways to produce food for a growing population in a changing world. This calls for extensive research efforts and the development of cross-disciplinary approaches.

Intensification of agricultural practices, climate alterations and the extensive exchange of plant material in the global market are associated with an increased risk of crop damages caused by pests and pathogens. In this context, maintaining a sufficient agricultural production largely relies on chemical control. An alternative is however the **use of cultivars that are genetically resistant to pests and diseases**. This provides an effective protection, does not imply any additional cost for producers, is environmentally friendly and safe for consumers. The development of resistant cultivars has therefore become a critical factor of competitiveness for breeders.

This strategy successfully protects a number of crops against their pests and diseases, but its main limit results from the selective pressures exerted on pathogen populations by plant resistances, which frequently favour the emergence of adapted strains, leading to resistance breakdown. Given the scarcity of resistance genes, there is an urgent need to improve the sustainability of these genetic resources. **A significant amount of research is still needed to better understand resistance mechanisms and the adaptive capacity of pathogens, to include that knowledge in resistance management models, and to develop efficient and sustainable management strategies. The necessary changes in agronomic practices will also raise a number of questions related to the acceptance level of such strategies by farmers, as well as socio-economic issues such as the coordination of actors in a given territory.**

In this context, the Institut National de la Recherche Agronomique (INRA), in the frame of its research metaprogramme “Sustainable Management of Crop Health”¹ (SMaCH), is holding an International Conference on the topic Plant Resistance Sustainability. Four sessions are devoted to (i) the impact of plant disease resistance on the structure and evolution of pathogen populations, (ii) sustainable and integrated breeding and deployment of genetic resistance, (iii) the exploitation of data on plant-pathogen molecular interactions to enrich and/or renew the strategies of sustainable management of plant resistance and, (iv) socio-economic issues related to the use of resistant varieties and their deployment in agro-systems. We hope the meeting will provide the opportunity for the scientists working in these topics to meet, discuss their work and establish new collaborations and perspectives.

The organising Committee would like to thank Chairs of the scientific sessions, invited speakers, and members of the Scientific Committee for their help in preparing the programme. We would also like to

¹ https://www.inra.fr/les_recherches/metaprogrammes/gestion_durable_de_la_sante_des_cultures

thank our sponsors for providing support to this meeting.

We hope you will enjoy the Plant Resistance Sustainability 2012 International Conference and your stay at La Colle-sur-Loup in Southern of France.

The Organising Committee

Organising committee

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 - **ROSSING Walter**, Wageningen University, Department of Plant Sciences, Wageningen, The Netherlands
 - **VAN DEN BOSCH Frank**, Rothamsted Research - Biomathematics and Bioinformatics Department, Harpenden, United Kingdom
-

Programme

Day 1 - Tuesday 16th October

- 11:00 a.m – 2:00 p.m. Registration Opening
- 2:00 p.m. Carole Caranta, INRA, France – Head of the Genetics and Plant Breeding Division
Welcoming address, presentation of the INRA's Metaprogramme "Sustainable Management of Crop Health" and of the Conference
- 2:20 p.m. OPENING LECTURE
Christopher C. Mundt, Oregon State University, United States of America
Sustainable management of plant resistance to pathogens and pests

Session 1: Impact of plant disease resistance on the structure and evolution of pathogen populations

Chairpersons: Bruce McDonald and Christian Lannou

- 3:05 p.m. Peter H. Thrall, CSIRO Plant Industry, Australia
INVITED SPEAKER (Key note)
The evolution of pathogen infectivity in natural and managed plant-pathogen systems
- 3:50 p.m. Sylvain Gandon, CNRS, France
INVITED SPEAKER (Key note)
Plant resistance and the evolution of pathogens
- 4:35 p.m. Romain Bourget, INRA, France
Dynamics of pathogen adaptation to an imperfect treatment
- 4:55 p.m. Coffee Break
- 5:25 p.m. Didier Andrivon, INRA, France
Erosion of quantitative resistance to *Phytophthora infestans*: from field observations to understanding population evolution
- 5:45 p.m. François Delmotte, INRA, France
Rapid adaptation to partial resistance in grapevine downy mildew: evidence from European populations of *Plasmopara viticola*
- 6:05 p.m. Marie-Claire Kerlan, INRA, France
Direct and indirect consequences of *Globodera pallida* selection by resistant plants
- 7:30 p.m. Dinner

Day 2 – Wednesday 17th October

Session 1: Impact of plant disease resistance on the structure and evolution of pathogen populations (cont.)

Chairpersons: Peter Thrall and Benoit Moury

- 9:00 a.m. Régine Delourme, INRA, France
Durability assessment of oilseed rape resistance to blackleg: nine years of recurrent selection of *Leptosphaeria maculans* populations in field experiment
- 9:20 a.m. Sophie Thomas, INRA, France
Effects of resistance combinations in melon on *Aphis gossypii* field populations
- 9:40 a.m. Yong-Ju Huang, University of Hertfordshire, United Kingdom
The race structure of *Leptosphaeria maculans* (phoma stem canker) in the UK
- 10:00 a.m. Fabien Halkett, INRA, France
Population genomic consequences of a major selection event in the poplar rust
- 10:20 a.m. Coffee Break
- 10:50 a.m. Thibault Leroy, INRA, France
When resistance genes introgressed in agrosystems act as gateways for virulent populations from the wild: the apple scab case study
- 11:10 a.m. Maree Horne, University of Southern Queensland, Australia
Virulence patterns, genetic diversity and population structure of Australian *Bipolaris sorokiniana* isolates

Session 4: Socio-economic issues related to the use of resistant varieties and their deployment in agro-systems

Chairperson: François Coleno

- 11:30 a.m. Philippe Baret, Earth & Life Institute, University of Louvain, Belgium
INVITED SPEAKER (Key note)
Path-dependency and socio-technical lock-in: the difficult quest of relevant innovations
- 12:30 a.m. Lunch

Session 2: Sustainable and integrated breeding and deployment of genetic resistance

Chairpersons: Chris Mundt and Alain Palloix

- 2:30 p.m. James Brown, John Innes Center, United Kingdom
INVITED SPEAKER (Key note)
Broad-spectrum disease resistance in crop varieties for an unpredictable future
- 3:15 p.m. Walter Rossing, Wageningen University, The Netherlands
INVITED SPEAKER (Key note)
Disease-suppressive landscapes – what role for spatial deployment?
- 4:00 p.m. Chris K. Sørensen, Aarhus University, Denmark
How effective is the durable resistance when encountering invasive strains? The case of wheat yellow rust pathogen *Puccinia striiformis*
- 4:20 p.m. Gustavo Azzimonti, INRA, France
Components of quantitative resistance to leaf rust in wheat cultivars
- 4:40 p.m. Julie Quenouille-Lederer, INRA, France
The durability of a major resistance gene is affected by quantitative trait loci which also confer quantitative resistance to virus
- 5:00 p.m. Caroline Djian-Caporalino, INRA, France
Experimental evidence of the efficiency of two resistance genes deployment strategies - pyramiding or alternating - for sustainable management of root-knot nematodes

1st Poster Session

- 5:20 p.m. Refreshment in front of the poster rooms
- 5:30 p.m. Poster Session
- 7:30 p.m. Dinner

Day 3 – Thursday 18th October

Session 2: Sustainable and integrated breeding and deployment of genetic resistance (cont.)

Chairpersons: Walter Rossing and Charles-Eric Durel

- 9:00 a.m. Frédérique Didelot, INRA, France
Sustainable control of apple scab obtained by the association of cultivar resistance, sanitation and soft chemical control
- 9:20 a.m. Dean Spaner, University of Alberta, Canada
Integrating the building blocks of agronomy and biocontrol into an IPM strategy for wheat stem sawfly
- 9:40 a.m. Christian Lannou, INRA, France
Exploring the potential of landscape diversification for limiting epidemic risk
- 10:00 a.m. Marjolein E. Lof, Wageningen University, The Netherlands
Durable plant disease resistance by evolution management - how?
- 10:20 a.m. Coffee Break
- 10:50 a.m. Frédéric Fabre, INRA, France
Sustainable plant resistance management in agricultural landscapes
- 11:10 a.m. Natalia Sapoukhina, INRA, France
Plant resistance deployment to control a diversified pathogen population: apple scab as a modelling case study

2nd Poster Session

- 11:30 a.m. Poster Session
- 1:00 p.m. Lunch

Session 3: From plant-pathogen molecular interactions to the durability of resistance

Chairpersons: Rients Niks and Carole Caranta

- 2:30 p.m. Laura Rose, Institute of Population Genetics, Germany
INVITED SPEAKER (Key note)
Molecular evolution between pathogens and wild tomatoes
- 3:15 p.m. Benoit Moury, INRA, France
INVITED SPEAKER (Key note)
Constraints on resistance and pathogenicity factors and their consequences on resistance durability
- 4:00 p.m. Beat Keller, University of Zurich, Switzerland
Analysis of the wheat *Lr34/Yr18* gene and the molecular basis of durable resistance
- 4:20 p.m. Marie-Hélène Balesdent, INRA, France
Molecular evolution of the *AvrLm7* avirulence gene of *Leptosphaeria maculans* under resistance gene selection in the field is driven by its genomic location, sexual reproduction and cropping practices
- 4:40 p.m. Coffee Break
- 5:10 p.m. Nathalie Boissot, INRA, France
Characterization of adaptation to a resistance gene with a pleiotropic effect, the *Vat* gene in melon
- 5:30 p.m. Sylvie German-Retana, INRA, France
Mutations in the Cylindrical Inclusion of *Lettuce mosaic virus* are associated with evolution towards resistance-breaking of eIF4E-mediated resistance in lettuce
- 5:50 p.m. Andrew Bent, University of Wisconsin, United States of America
Durable soybean cyst nematode resistance due to multiple copies of a multi-gene block at *Rhg1*
- 6:10 p.m. Bruno Favery, INRA, France
Loss-of-susceptibility as an alternative for pathogen resistance
- 6:30 p.m. Laurence Albar, IRD, France
A mutation in a homologous of CPR5 is associated to resistance to Rice yellow mottle virus in rice
- 8:30 p.m. Reception Dinner

Day 4 – Friday 19th October

Session 4: Socio-economic issues related to the use of resistant varieties and their deployment in agro-systems

Chairpersons: François Coleno and Marion Desquilbet

9:00 a.m. Marion Desquilbet, INRA, Toulouse School of Economics, France

INVITED SPEAKER (Key note)

Pest Resistance Management: an economic perspective

9:45 a.m. Stéphane Lemarié, INRA, France

Resistance management and information acquisition: an economic analysis

10:05 a.m. François Hochereau, INRA, France

Cognitive alignment, Accommodation and Percolation: three ways of extension of resistant varieties and sustainable farming methods

10:25 a.m. Coffee Break

10:55 a.m. Laure Hossard, INRA, France

Spatial cultivar deployment and residue management: What impact for phoma stem canker of winter oilseed rape and resistance sustainability?

11:15 a.m. Lydia Bousset, INRA, France

Controlling cyclic epidemics on the crops of the agro-ecosystems by resistant varieties: when should one simplify the reality?

11:35 a.m. Olivier Le Gall, INRA, France – Director of the INRA's metaprogramme "Sustainable Management of Crop Health" - Head of the Plant Health and Environment division

Conclusion and perspectives

12:00 a.m. End of the "Plant Resistance Sustainability" Conference

Poster sessions

1. Session 1: Impact of plant disease resistance on the structure and evolution of pathogen populations

- 1.1. Lê Van A.; Caffier V.; Lasserre-Zuber P.; Chauveau A.; Brunel D.; Le Cam B.; Durel C.E.
Differential selection pressures exerted by resistance QTLs on a pathogen population: a case study in apple - *Venturia inaequalis* pathosystem
- 1.2. Blanchard D.; Monod H.; Lannou C.; Goyeau H.
Modeling crop disease risk and variety-pathotype interactions at the regional scale by a Bayesian approach
- 1.3. Wicker E.; Lebeau A.; Pensec F.; Daunay M-C.; Dintinger J.
New insights on virulence and evolutionary dynamics of the *Ralstonia solanacearum* species complex? Keys and challenges in the search of durable resistance in *solanaceae*
- 1.4. Zenbayashi-Sawata K.; Kito H.; Ashizawa T.; Koizumi S.
Durability of the partial resistance to blast controlled by several major QTL in upland rice and efficacy of the introgression of resistance genes from upland to paddy rice cultivar
- 1.5. Frey P.; Xhaard C.; Barrès B.; Fabre B.; Halkett F.
Impact of host domestication on the genetic structure of the poplar rust fungus
- 1.6. Kuhn M-H.; Massire A.; Cantet M.; Damidaux R.; Gibowski E.; Ruellan Y.; Lefebvre V.; Panabières F.
Contribution of microbial core-collections and exploitation of polygenic resistances to the development of *Phytophthora* control strategies in solanaceous crops
- 1.7. Caffier V.; Lasserre P.; Giraud M.; Lascostes M.; Le Cam B.; Durel C.E.
Erosion of quantitative resistance loci related to an increase in pathogen aggressiveness and consequences on the efficiency of their combination with a major gene
- 1.8. Reza Ahmadi M.; Javan-Nikkhah M.; Ali Aghajani M.A.; Reza Naghavi M.
Pathogenicity of genetically different *Sclerotinia sclerotiorum* isolates on important crops
- 1.9. Basler R.; Edwards S.; Thomas J.
Genetic structure and chemotype composition of *F. graminearum* in forage maize in the United Kingdom
- 1.10. Kydralieva K.A.; Mamytova B.A.; Muratov V.S.; Khudaibergenova B.M.; Jorobekova Sh.
Domestication of *Rhizobacteria* as biocontrol agents
- 1.11. Mitrousia G.K.; Huang Y-J.; Hall A. M.; Fitt B. D. L.
Analysing virulent and avirulent *Leptosphaeria maculans* isolates to understand R gene-mediated resistance in *Brassica napus*
- 1.12. Le May C.; Onfroy C.; Moussart A.; Tivoli B.; Andrivon D.; Baranger A.; Pilet-Nayel M.L.
Impact of crop successions including legumes species on the durability of partial resistance to *Aphanomyces euteiches* in pea

2. Session 2: Sustainable and integrated breeding and deployment of genetic resistance

- 2.1. Patocchi A.; Auwerkerken A.; Masny S.; Nybom H.; Pillion V.; Tamm L.; Vávra R.; Andreas Peil A.; Padder B. A.; Rühmer T.
VINQUEST: The monitoring of *Venturia inaequalis* virulences initiative
- 2.2. Barbary A.; Palloix A.; Fazari A.; Marteu N.; Castagnone-Sereno P.; Djian-Caporalino C.
Importance of the genetic background for sustainable resistance: experimental evidence for a major resistance gene to nematodes
- 2.3. Rodemann B.
Differentiation in the susceptibility of German winter wheat cultivars against *Mycosphaerella graminicola* isolates under controlled and field conditions
- 2.4. Maisonneuve B.; Martin E.; Jean L.; de Vallavieille-Pope C.; Pitrat M.
Effects of lettuce cultivar mixtures, differing for resistance genes, on *Bremia lactucae* incidence
- 2.5. Djian-Caporalino C.; Palloix A.; Navarrete M.; Lefevre A.; Mateille T.; Védie H.; Goillon C.; Trottin Y.; Boniol P.; Castagnone-Sereno P.
The GEDUNEM project: Varietal and technical innovations for the sustainable and integrated management of root-knot nematodes in protected vegetable cropping systems
- 2.6. Seguin M.; Garcia D.; Mattos C.; Doaré F.; Le Guen V.
Genetic (QTL) mapping approach revealed unexpected patterns of bypassed and sustainable resistances to *Microcyclus ulei* in rubber tree (*Hevea* spp.)
- 2.7. Pilet-Nayel M-L.; Hamon C.; Lesné A.; Le May C.; Baranger A.; Tivoli B.; Moussart A.
Combining polygenic resistance with prophylactic and cultural methods for sustainable management of *Aphanomyces* root rot in pea
- 2.8. Sutherland M. W.; Knight N. L.; Martin A.
The characteristics of resistance to *Fusarium* crown rot in wheat
- 2.9. Tereshonkova T.A.; Gorshkova N.S.; Emelina, M.N.; Khovrin A.N.; Ignatova S.I.
Use of genes for resistance to powdery mildew of tomato from various sources in order to create genotypes with durable resistance
- 2.10. Ziemichod M.E.; Arseniuk E.
Comparison of winter wheat and triticale cultivars in the seedling stage and adult plant stages to *Stagonospora nodorum*
- 2.11. Zamani-Noor N.; Rodemann B.
Effect of climate conditions and frost damage on the occurrence of grey mould disease caused by *Botrytis cinerea* in oilseed rape fields
- 2.12. Aitkhozhina N.
Brown rot fungus *Monilia fructigena* in the centre of apple origin
- 2.13. Amro M.A.; Abd El-Rahim G.H.; Abd El-Raheem A.A.
Population fluctuations, relative susceptibility and control of *Thrips tabaci* (Lind.) on some onion and garlic cultivars and strains
- 2.14. Van Ghelder C.; Khallouk S.; Polidori J.; Duval H.; Kleinhentz M.; Dirlewanger E.; and Esmenjaud D.

Biological features, positional cloning and validation of the Ma gene for high-level and complete-spectrum resistance to root-knot nematodes in *Prunus*

- 2.15. Ashizawa T.; Nakabayashi J.; Sasaki A.; Ishikawa K.; Sasahara M.; Tsukamoto S.; Koga H.; Watanabe T.; Hirayae K.
Simulation of race dynamics assists durability of blast resistance in multilines in Japan

3. Session 3: From plant-pathogen molecular interactions to the durability of resistance

- 3.1. Vigne E.; Komar V.; Marmonier A.; Demangeat G.; Fuchs M.; Lemaire O. Management of the grapevine fanleaf degeneration: are innovative and new control strategies environmentally sound?
- 3.2. Gravot A.; Wagner G.; Lariagon C.; Delourme R.; Manzanares-Dauleux M-J. Deciphering the functional typology of resistance QTL through metabolomics
- 3.3. Garcia D.; Koop D.M.; Pujade-Renaud V.; Guyot J.; Mattos C.R.R.; Seguin M.; Le Guen V. Long lasting rubber tree resistance to *Microcyclus ulei*
- 3.4. Ziemichod M. E.; Arseniuk E.
Effect of androgenesis of wheat and triticale on *Stagonospora nodorum* blotch (SNB) resistance
- 3.5. Körösi K.; Bán R.; Baglyas G.; Virányi F.
Induced resistance in sunflower: defense mechanisms against a biotrophic and a necrotrophic plant pathogen
- 3.6. Nasar V.; Fengming S.
Arabidopsis Mitogen Activated Protein Kinase 4 and its homologs positively regulate defense responses against *Botrytis cinerea* in different plant species
- 3.7. Singh N.
Expression of pathogen *Fusarium moniliforme* and its biochemical response in early stages of maize (*Zea mays* L.)
- 3.8. Fraire-Velázquez S.; Ramírez-Cabral N.; De La Cruz-Rodríguez Y.; Vázquez-Herrera C.
In search of anthracnose susceptibility host factors in common bean
- 3.9. Madduri M.
Tracing genes for resistance to apple scab disease *Venturia inaequalis*
- 3.10. Pichon M.; Gascuel Q.; Martinez Y.; Munos S.; Pouilly N.; Blanchet N.; Boniface M. C.; Godiard L.; Vincourt P.
Sunflower downy mildew: Microscopic and imagery studies of *Plasmopara halstedii* infection, inventory of pathogenicity effectors and map-based cloning of a quantitative disease resistance locus
- 3.11. Corio-Costet M.F.; Lambert C.; Cluzet S.; Merdinoglu D.; Dufour M.C.
BioMolChem, a tool to assess the defence status of grapevines after stimulation, or not, of cultivar or resistant genotypes: from genes to the field
- 3.12. Garbaczewska G.; Chouda M.; Otulak K.
Ultrastructural studies the participation of organelle in the TRV infection of tobacco and potato
- 3.13. Otulak K.; Garbaczewska G.

The ultrastructural events in hypersensitive and compatible reactions of potato and tobacco infected with PVY necrotic strains

- 3.14. Le-Berre J-Y.; Gourgues M.; Morabito C.; Keller H.; Panabieres F.; Attard A.
Genome-wide transcriptome analysis of the *Arabidopsis thaliana*/*Phytophthora parasitica* interaction: towards the characterization of genes involved in plant susceptibility to soilborne oomycetes
- 3.15. Allasia V.; Ponchet M.; Panabières F.; Keller H.
An *Arabidopsis thaliana* glycosyltransferase contributes to downy mildew disease
- 3.16. Evangelisti E.; Minet-Kebdani N.; Attard A.; Kuhn M-L.; Panabières F.; Gourgues M.
Four secreted proteins transiently accumulated during the penetration process of *Phytophthora parasitica* facilitate plant infection
- 3.17. Lecomte Ph.; El Hajj Assaf Ch.; Da Rocha M.; Chetouhi Ch.; Danchin E.; Langin T.
Identification of effectors secreted by the fungal pathogen *Fusarium graminearum*, main causal agent of FHB on wheat
- 3.18. Hok S.; Allasia V.; Naessens E.; Ribes E.; Andrio E.; Danchin E.; Panabières F.; Attard A.; Voll L.; Saur A.; Hückelhoven R.; Grill E.; Marco Y.; Barlet X.; Clément M.; Keller H.
Oomycete and fungal pathogens recruit a plant receptor to establish disease in *Arabidopsis thaliana*
- 3.19. Gorovits R.; Kolot M.; Moshe A.; Sobol I.; Czosnek H.
Correlation between coat protein aggregation and resistance of tomato to *Tomato yellow leaf curl virus* (TYLCV)

4. Session 4: Socio-economic issues related to the use of resistant varieties and their deployment in agro-systems

- 4.1. Garcia A.V.; Forzani C.; Yoshimoto K.; Balagué C.; Bueso E.; Colcombet J.; Hirt H.
The AGC2 kinase OXI1 regulates salicylic acid-dependent plant immunity and cell death programs in *Arabidopsis*
- 4.2. Adedotun M.
The farmers view about the use of different agricultural crop varieties in the Federal capital territory of Abuja Nigeria

Abstracts of Oral presentations

Opening session

Sustainable Management of Plant Resistance to Pathogens and Pests

Mundt C. C.

Dept. of Botany and Plant Pathology, 2082 Cordley Hall, Oregon State Univ., Corvallis, OR 97331-2902, USA

Abstract

Genes for resistance to pathogens and pests unquestionably represent one of the world's most precious resources. Increased demand for food and fiber, coupled with increasing environmental issues, make sustainable use of this resource especially crucial. The field of durable resistance has thankfully matured beyond claims for superiority of one approach to durability over another. Multiple mechanisms and multiple types of gene combinations likely protect plants in natural systems, and there is now broad recognition that many approaches and strategies have a potential role to play in managed ecosystems.

This talk will focus on what has been learned about resistance durability in recent years, as well as the questions that still remain. Knowledge of the molecular mechanisms underpinning major gene interactions has advanced substantially in the past ten years, potentially contributing to both breeding for resistance and improved understanding of virulence impacts on pathogen fitness. Though the molecular basis of partial resistance is less clear, substantial evidence has accumulated for the relative simplicity of inheritance and, thus, ease of use in breeding programs. There also is increasing evidence for specific interactions with partial resistance, though implications of this specificity for resistance durability are still relatively unknown. Mechanisms by which resistance gene pyramids contribute to durability remain elusive, though intriguing ideas have been generated for identifying gene combinations that may be most durable. Cultivar mixtures and related approaches to diversification have now been used successfully with an increasingly broad range of pathosystems. Identifying the diseases and conditions that are most conducive to the use of diversity has been surprisingly difficult, and it also is clear that the selective influence of diversity on plant pathogen populations is a much more complex process than previously expected. The importance of considering resistance durability in a landscape context has received increasing emphasis and is an important future area of research. Experimental evidence is beginning to accumulate to test the logical conclusion that integration of different control methods, both genetic and non-genetic, should increase the epidemiological impacts and durability of host plant resistance.

Advances in molecular marker technology have contributed to both an understanding of the underlying genetics of resistance, and to implementation of resistance breeding. The value of markers for identifying major genes is quite clear and will provide major benefits, e.g., in constructing resistance gene pyramids. A plethora of QTL studies of partial resistance have been reported in recent years, with the hope of contributing to breeding for quantitative resistance. The actual impact of these studies will depend greatly on the degree to which the identified QTL are expressed in different genetic backgrounds, a question that has not yet been adequately answered. Transgenic approaches will likely provide opportunities for control of some recalcitrant pathogens, though issues of durability for transgenes are likely to be no different than other genes for resistance. Advances in molecular approaches have not replaced the need high quality phenotypic analysis and field screening methodologies, and field-based studies are likely to remain of signal importance in the foreseeable future.

Session 1
Impact of plant disease resistance
on the structure and evolution
of pathogen populations

The evolution of pathogen infectivity in natural and managed plant-pathogen systems

Thrall P. H.; Burdon J. J.

CSIRO Plant Industry - Black Mountain, Black Mountain Laboratories Clunies Ross Street, Black Mountain ACT 2601, Australia

Abstract

Antagonistic coevolution is a critical force driving the evolution of host and pathogen diversity, yet the selective processes underpinning reciprocal adaptive changes are still not well understood. Local adaptation studies demonstrate partner impacts on fitness and adaptive change, but do not directly expose genetic processes predicted by theory. Thus, we have little knowledge of the relative importance of fluctuating selection vs. arms-race dynamics in maintaining polymorphism in host-pathogen interactions or how these are impacted by host resistance structure, pathogen life-history, and spatio-temporal environmental variation. We discuss these issues with a particular focus on pathogen infectivity using illustrative examples from both natural and agricultural systems and then focus in more detail on the wild association between *L. marginale* and *M. lini* which we have studied for more than 20 years. Extensive cross-year epidemiological, infection and genetic studies of multiple wild host and pathogen populations demonstrate asynchronous phenotypic fluctuations in resistance and infectivity among demes. Importantly, changes in allelic frequencies at pathogen infectivity loci, and in host recognition of these genetic variants, correlate with disease prevalence during natural epidemics. These data strongly support reciprocal coevolution maintaining balanced resistance and infectivity polymorphisms, and highlight the importance of characterising spatial and temporal dynamics in antagonistic interactions. We conclude with some general comments and highlight some future research directions. In particular, we argue that there is a strong need for the application of eco-evolutionary principles to managing host-pathogen interactions in agro-ecological systems.

Keywords: Disease, coevolution, virulence, host-pathogen, resistance

Plant resistance and the evolution of pathogens

Gandon S.

Centre d'Ecologie Fonctionnelle et Evolutive, Unité Mixte de Recherche, Montpellier Cedex 5, France

Abstract

The benefits associated with the creation of new plant varieties is constantly threatened by the evolution of pathogens (i.e. pathogen resistance to plant resistance). New pathogens can appear by mutation, migration and/or recombination and spread in fields of resistant plants. Is it possible to limit this evolution? To answer this question it is useful to look beyond the specificities of plant-pathogen interactions. I will use general epidemiological models to look at the effect of various factors on the evolution of pathogens. First, I will focus on a treatment strategy where the use of resistant plants may vary in space to prevent the rise of pathogen evolution. There is a critical size of the area planted with the resistant variety, under which the pathogen-resistant strain cannot persist. Second, I will focus on the evolution of pathogens on multiple loci. The analysis of these models reveals the importance of feedbacks between the epidemiological and evolutionary dynamics. These models illustrate how these feedbacks can be exploited to control pathogen evolution.

Keywords: Pathogen evolution, epidemiology, population genetics, transmission, cost of resistance

Dynamics of pathogen adaptation to an imperfect treatment

Bourget R.^{1,2}; Chaumont L.¹; Sapoukhina N.²

¹LAREMA, Université d'Angers, UFR Sciences

²IRHS (INRA, Université d'Angers, Agrocampus Ouest), SFR QUASAV

Abstract

The use of treatments conferring host resistance greatly affects the pathogen dynamics and evolution. A particular type of treatments like imperfect vaccines, low pressure drugs or quantitative trait loci can induce partial resistance of the hosts. It is believed that, reducing within-host growth rate and the probability of infection, the imperfect treatments decrease pathogen abilities to evolve.

Even if the detection of pathogen adaptation is currently difficult, it seems to be realized by a succession of mutations that restoring progressively pathogen fitness. It was also been noticed that the time of the pathogen adaptation could depend on the treatment properties. The evolutionary consequences of the imperfect treatments have been studied with theoretical approaches, especially in the medical field. Nevertheless, there is currently no theoretical framework describing the continuous process of pathogen fitness restoration.

The objective of this work is to study the dynamics of the pathogen adaptation to a host receiving an imperfect treatment and thereby to estimate the treatment durability. We developed and analyzed a stochastic model accounting for the birth, mutation, death events and competitive interactions between pathogen individuals over a continuous time. Moreover, the model takes into account the fitness differences among pathogen individuals, caused by mutations. In numerical simulations we varied the values of pathogen growth rate and the shape of the mutation law to determine the time until the complete pathogen adaptation. We discuss the application of our theoretical results to design of treatment strategies slowing down pathogen evolution.

Keywords: Imperfect treatment, pathogen evolution, stochastic model

Erosion of quantitative resistance to *Phytophthora infestans*: from field observations to understanding population evolution

Andrivon D.; Corbière R.; Glais I.; Montarry J.; Pasco C.; Marquer B.

INRA, Agrocampus Rennes, UMR1099 BiO3P, F-35653 Le Rheu, France

Abstract

Quantitative resistance is generally assumed to impose low selective constraints on pathogen populations, and hence to be durable. This supposes that quantitative resistance is based on life history traits for which either limited variability exists among pathogen genotypes and /or existing variation is not specific to one source of resistance, resulting in an absence of local adaptation even where quantitative resistance is used. However, pathogenicity analyses on populations of the potato late blight pathogen *Phytophthora infestans* show 1) that extensive variability exists for each of the pathogen life history traits related to quantitative pathogenicity (such as latent period duration, sporulation capacity or lesion growth rate), and 2) that quantitative resistance in the field results from combinations of alterations of pathogen life history traits, roughly independent from one another (Clement *et al.*, 2010). This makes specific adaptation to one or more components of quantitative resistance possible, provided selection is maintained by the widespread use of the same resistant cultivar (Andrivon *et al.*, 2007). Nevertheless, this pathosystem is also characterized by strong gene flow and cultivar deployment over large regional areas, which cause population to adapt to dominant host cultivars at regional or national scales (Montarry *et al.*, 2006, 2008), irrespective of their initial resistance level. This explains why quantitative resistance can be eroded when cultivars with this type of resistance are used over large acreages and long periods of time, but remains stable against populations mainly selected by susceptible cultivars and/or genetically isolated (Forbes *et al.*, 2004).

Keywords: late blight, aggressiveness, adaptation, partial resistance, trade-offs

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Rapid adaptation to partial resistance in grapevine downy mildew: evidence from European populations of *Plasmopara viticola*

Rouxel M.^{1,2}; Mestre P.²; Delière L.¹; Delmotte F.¹

¹INRA, ISVV, UMR1065 Santé et Agroécologie du Vignoble, F-33883 Villenave d'Ornon, France

²INRA, UMR1131 Santé de la Vigne et Qualité du vin, F-68000 Colmar, France

Abstract

Agro-ecosystems provide one of the most convincing examples of situations where anthropogenic effects are major determinants of biotic interactions between host and parasites (Thrall et al. 2010). Recent studies have demonstrated intensive farming practices are likely to select for fast-growing and more virulent parasites (Mennarat et al. 2011, Pulkkinen et al. 2010). It is therefore possible to integrate knowledge in evolutionary biology to understand plant diseases and to improve pest management in agro-ecosystems. The use of crops showing resistance to fungal diseases is a major modification of agro-ecosystems that is now accelerated by advances in marker technologies used in breeding strategies. Quantitative resistances create new conditions for pathogen growth and transmission that are different from those experienced by parasites on susceptible plants. It is therefore expected that plant disease resistance might alter selection on life-history traits and modify the adaptive strategy of the pathogens. Confirming this view, plant pathogens have been shown to present differential adaptation to host cultivars, sometimes leading to erosion of quantitative resistance (Andrison et al., 2007; Mundt et al. 2008; Pariaud et al. 2009).

Plasmopara viticola, the causal agent of grapevine downy mildew, is an obligate biotrophic Oomycete. It was introduced from North America into Europe in the late 1870s, probably with the importation of American rootstocks resistant to Phylloxera. European grapevine (*V. vinifera*) revealed to be highly susceptible to the disease, and the current strategy of control relies on the use of fungicide treatments. The use of resistant grapevine varieties is a promising component of a sustainable management strategy of grapevine downy mildew. However, since all *V. vinifera* cultivars are susceptible to *P. viticola*, the resistance needs to be introduced from other *Vitis* species through breeding programmes that ensure also the maintenance of important agronomic characteristics. In

Europe, conventional breeding programmes for resistance to grapevine pathogens have resulted in the creation of varieties that are currently grown on limited acreages, such as Regent and Solaris. However, the first breakdown of resistance to grapevine downy mildew has recently been reported for a variety containing a major QTL of resistance (Peressotti et al. 2010). It is therefore important to determine to what extent *P. viticola* populations can be selected for increased aggressiveness by grapevine cultivars with different levels of partial resistance.

In this study, we aimed to determine (i) whether populations of *P. viticola* infecting partially resistant varieties have adapted to these resistances, (ii) whether these populations were more aggressive than populations from *V. vinifera* (life-history traits assessed on a susceptible host). We collected 24 isolates of *P. viticola* in European vineyards on *V. vinifera* varieties (n=8), as well as on six commercial varieties showing partial resistance to grapevine downy mildew (n=16). Cross-inoculations experiments were conducted in which the 24 isolates were compared for their aggressiveness on *V. vinifera* (cv Cabernet sauvignon) and on the six resistant varieties. Aggressiveness was measured through a variety of quantitative traits expressed during the host–pathogen interaction. These traits, referred to as life history traits, were: latent period, spore production rate and spore size. Our results showed evidence for significant increase in aggressiveness of populations of *P. viticola* collected on partially resistant varieties: Compared to the population of isolates coming from *V. vinifera*, the latent period reveals to be shorter, sporulation higher and mean sporangia size smaller, indicating a different adaptive strategy for these isolates. These results bring evidence that the deployment of these partially resistant varieties will likely result in an increased aggressiveness of *P. viticola* isolates. It is therefore a possibility that long-term evolution of pathogen populations may challenge the durability of these grapevine partially resistant varieties if they are used alone (Calonnec *et al.* in press). Combined strategies based on both natural resistance and other control methods (biological control, sanitation, fungicide) could be a valuable research avenue with the aim to provide a more sustainable management of grapevine downy mildew.

Keywords: Partial disease resistance, aggressiveness, oomycetes, downy mildew, grapevine

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Selection by resistant plants

Kerlan M.C.¹, Montarry J.², Renault L.², Dantec J.P.¹ Rouaux C.²; Fournet S.²

¹ INRA, UMR 1349 IGEPP, Domaine de Keraïber, F-29260 Ploudaniel

² INRA, UMR 1349 IGEPP, Domaine de la Motte, BP35327, F-35653 Le Rheu Cedex

Abstract

The environment could impact the adaptation of a parasite to its host. Here we focused on the cost due to trade-offs between life history traits in the case of the pathosystem cyst nematode *Globodera pallida* and resistant potato genotypes. We explored if durability was variable in different resistant potato genotypes harboring the same resistance QTL but differing by their genetic background. We also investigated the indirect consequences of the resistance adaptation in term of local adaptation and cross-virulence. Following the virulence of the potato cyst nematode *G. pallida*, through experimental evolution, our results showed that nematode populations were able to adapt to the resistance of three potato genotypes carrying the *GpaV* QTL from *Solanum vernei* and that the plant genetic background impacts the resistance durability. The pattern of local adaptation observed here indicates that the process of divergent selection has occurred during the experimental evolution performed from the same initial nematode population, and that there was a trade-off between the adaptation to a resistant potato genotype and the adaptation to another one. In term of cross-virulence between potato genotypes derived from different resistance origins (*S. sparsipilum* and *S. spgazzinii*), we showed that the adaptation to resistance QTL *GpaV_{vrn}* does not necessarily allow the adaptation to collinear *GpaV* loci. Results presented here could be used 1) to predict evolution of nematode populations in natural agro-ecosystems and to identify durable strategies of resistance's deployment 2) to design new genotypes carrying different combinations of genetic factors to increase their durability.

Keywords: nematode, potato, resistance, sustainability, trade-offs, local adaptation, cross-virulence

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Durability assessment of oilseed rape resistance to blackleg: Nine years of recurrent selection of *Leptosphaeria maculans* populations in field experiment

Brun H.¹; Fudal I.²; Ermel M.¹; Besnard A.L.¹; Bousset L.¹; Marquer B.¹; Eber F.¹; Renard M.¹; Chèvre A.M.¹; Delourme R.¹

¹ INRA, UMR1349 IGEPP, F-35653 Le Rheu, France

² INRA, UR1290 BIOGER-CPP, F-78850 Thiverval Grignon, France

Abstract

Leptosphaeria maculans is the fungus responsible for blackleg disease that threatens oilseed rape crops worldwide. Two kinds of resistance can reduce damages on yield: non durable qualitative resistance based on gene for gene relationships and considered more durable polygenic quantitative resistance that mainly limits the development of stem base canker (Delourme et al., 2006).

The effect of quantitative resistance of oilseed rape on durability of a major resistance gene, *Rlm6*, towards *L. maculans* was assessed in multi-year field experiment to simulate commercial use. Two pairs of near isogenic lines were produced by introducing *Rlm6* into two genetic backgrounds, EuroI (susceptible) and Darmor (quantitative resistance) named EuroIMX and DarmorMX, respectively. Each year, 4 separate field trials were established and each inoculated with fungal populations selected recurrently on one of the 4 genotypes. The results obtained show that in both trials where EuroI and Darmor residues were used recurrently similar ranking of genotypes for resistance/susceptibility were observed. Effectiveness of Darmor resistance at stem level fluctuated according to the disease pressure of the year but it was always significantly higher than that of EuroI. The major gene *Rlm6* confers a high level of resistance to *Leptosphaeria maculans* but not durable when introduced into a susceptible oilseed rape genetic background. In both trials where residues of *Rlm6* lines were used, the size of fungus population dramatically decreased in the 2nd year. EuroIMX resistance was overcome in the 3rd year when the primary inoculum was recurrently selected on EuroIMX. On the contrary, when *Rlm6* was combined with quantitative resistance from Darmor the effectiveness of resistance remained high. These results suggest a much slower adaptation and multiplication of the virulent isolates on the DarmorMX line and therefore indicate a strong effect of the polygenic quantitative resistance on the durability of *Rlm6* resistance (Brun et al, 2010).

The inoculation of stem in controlled conditions with isolates obtained from the four lines did not show an increase of the aggressiveness on the susceptible line EuroI or on the resistant line Darmor, which suggests there was no adaptation to quantitative resistance after three years of recurrent selection on Darmor or DarmorMX lines in the field. The results of the aggressiveness on stem of isolates taken from the four lines after eight or nine years of recurrent selection will be discussed. Comparison of mutational events at *AvrLm6* locus of isolates collected on Darmor, EuroI, DarmorMX and EuroIMX after three, eight and nine years of recurrent selection will also be presented.

Keywords: *Leptosphaeria maculans*, oilseed rape, resistance durability, field experiment, resistance combination

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Effects of resistance combinations in melon on *Aphis gossypii* field populations

Thomas S.¹; Vanlerberghe F.¹; Chareyron V.²; Mistral P.²; Boissot N.²

¹INRA, UMR CBGP, Campus international de Baillarguet, CS 30016, F-34988 Montferrier-sur-Lez, France

²INRA, UR1052, Génétique et Amélioration des Fruits et Légumes, B.P. 94, F-84143 Montfavet cedex

Abstract

Host-plant resistance has proven its efficiency in controlling pests, including aphids which are one of the major hemipteran pests in crops. Nevertheless, resistance-breaking biotypes have occurred in several plant-aphid systems. The aim of our field study was to investigate the impact of distinct combinations of aphid-resistance *Vat* gene and QTLs in melons on the density and genetic structure of field populations of the melon aphid species *Aphis gossypii*. We set up field trials with melons combining (i) resistance alleles both at the *Vat* locus and at QTL (*Vat*/QTL), (ii) resistance allele at the *Vat* locus and susceptible alleles at QTL (*Vat*/-) or (iii) susceptible allele at the *Vat* locus and resistant alleles at QTL (-/QTL). All combinations had a homogenous genetic composition except at the *Vat* locus and QTL (Boissot et al 2010). Twelve trials were conducted in three distant melon producing areas, Southeastern France (SE), southwestern France (SW) and French West Indies (FWI), to take into account that aphid populations are geographically structured (Thomas et al 2012).

Aphid population densities were significantly lower on *Vat*/QTL melon plants than on -/QTL plants over trials, revealing a strong effect of the *Vat* gene *in situ*. Conversely, no effect of the QTL was observed on aphid density. We also collected about 2800 aphids from these trials that were distributed in two classes whether they were found as isolated apterous individuals on the leaf soon after melon plantation or whether they were collected from a colony later on. Each aphid was genotyped for eight microsatellite loci. Whatever the resistance combination and area, the genetic diversity decreased dramatically during the melon development, from isolated apterous to colonies, which illustrated a strong host plant selective pressure. We also noticed a *Vat* gene effect in reducing the genetic diversity of both apterous populations in SE and colonies in SE and SW. Furthermore, according to a genetic clustering analysis, the 138 multilocus genotypes identified were separated in two clusters called the NM1 cluster and the Cucurbit cluster. Aphids from the NM1 cluster were significantly more affected by the *Vat* gene as apterous individuals were less numerous on *Vat*/QTL than on -/QTL melons and they did not develop into colonies on *Vat*/QTL and on *Vat*/- melons.

In conclusion, this field study did not provide experimental evidence that the combination of qualitative effective major gene and quantitative polygenic resistance to *A. gossypii* in melon improves control of the aphid populations. QTLs had no effect on demography or on clonal diversity and structure of aphid populations. On the contrary, the effect of the major resistance gene *Vat* was shown to depend on the clonal diversity and structure of the aphid populations. However, this clonal diversity might substantially vary in time and space according to many ecological factors, which could prevent or at least slow down the spread of a resistance breaking biotype.

Keywords: *Cucumis melo*, Aphid resistance, host plant selection

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The race structure of *Leptosphaeria maculans* (phoma stem canker) in the United Kingdom

Yong-Ju H.; Georgia K. Mitrousia G.K.; Fitt B. D.L.

School of Life Sciences, University of Hertfordshire, Hatfield, Herts, AL10 9AB, UK

Abstract

Phoma stem canker (*Leptosphaeria maculans*) is the main disease problem on winter oilseed rape (*Brassica napus*) in the UK, causing losses of > £100M p.a., despite use of fungicides. Two types of resistance to *L. maculans* have been identified in *B. napus*. These are major resistance (*R*) gene-mediated qualitative resistance that operates in cotyledons and leaves in autumn (Balesdent et al., 2001) and quantitative resistance that operates in leaf stalk and stem tissues, after initial leaf infection until harvest in summer (Huang et al., 2009). *R* gene-mediated resistance to *L. maculans* is associated with a gene-for-gene interaction in which the product of a pathogen effector (*Avr*) gene is recognised by the product of a host *R* gene so that the pathogen is unable to infect the host. Therefore, *R* gene-mediated resistance to *L. maculans* is effective in protecting plants only if the corresponding avirulent allele is predominant in the local *L. maculans* population (Balesdent et al., 2001; Rouxel et al., 2003). In *Brassica* spp., 15 *R* genes (*Rlm1-Rlm10*, *RlmS*, *LepR1-LepR3*, *BLRM1-2*) that confer resistance against *L. maculans* have been identified. It is important to monitor the races in *L. maculans* populations since this monitoring can provide information to guide deployment of cultivars with different *R* genes to increase their durability.

Winter oilseed rape field experiments at eight different sites in the UK with the cultivar Drakkar (no *R* gene, used as trap for all pathogen races) were used to sample the races of *L. maculans* at those sites. Leaves with phoma leaf spots were collected in autumn/winter 2010/2011 and single pycnidial isolates were obtained. From each site, 15 to 30 isolates were collected, with 296 isolates in total. To determine the race of each of the isolates, a set of differential cultivars/lines with known *R* genes was used for cotyledon inoculation tests. To date, 57 isolates have been characterised by cotyledon inoculation. The results show that the avirulent allele of *AvrLm7* is predominant at all the sites sampled, with a mean frequency of 100%. This indicates that the corresponding resistance gene *Rlm7* is effective. The frequencies of *AvrLm5* (98%) and *AvrLm6* (93%) avirulent alleles were greater than those of *AvrLm4* (53%) and *AvrLm1* (23%). The populations of *L. maculans* were 100% virulent at *AvrLm2*, *AvrLm3* and *AvrLm9* loci.

There were differences between sites in frequency of *Avr* alleles. For example, frequencies of *AvrLm4* in Norfolk and Shropshire were 100% and 90%, respectively, which were greater than those at other locations (mean frequency 30% at Rothwell, Stockbridge and Cowlinge).

In addition, single pycnidial isolates of *L. maculans* were obtained from cultivars with quantitative resistance (with no known *R* gene) (Es-Astrid and NK Grandia). The frequencies of *Avr* alleles were investigated by cotyledon inoculation. This was done to investigate whether there are effects of host background (quantitative) resistance on selection for *Avr* alleles. It was found that there are some host selection effects; for example the frequency of the avirulent allele *AvrLm7* was 100% in populations obtained from Drakkar but 70% in populations obtained from cultivars with quantitative resistance. For further analysis, the isolates were cultured to harvest mycelium for DNA extraction and molecular characterisation.

Keywords: *Leptosphaeria maculans*, *Brassica napus*, *R* gene-mediated resistance, durability of resistance

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Population genomic consequences of a major selection event in the poplar rust.**De Mita S.; Persoons A.; Frey P.; Duplessis S.; Halkett F.**

INRA, Université de Lorraine, UMR1136 Interactions Arbres - Microorganismes, F-54280 Champenoux, France.

Abstract

While the rapid pace of pathogen evolution represents a major impediment to sustainable agriculture, it also provides interesting opportunities to better understand the evolutionary biology of host-parasite interactions. The field of population genomics has recently emerged at the cross between traditional population genetics and the development of high-throughput techniques, with the aim to decipher the strength of selection events on genome evolution. Although not yet widely applied to plant pathology studies, these approaches seem particularly well suited to reveal the genetic bases of the adaptive potential of fungal plant pathogens.

We focused on the poplar rust, which has become a leading international model in forest pathology since the completion of both the host (*Populus trichocarpa*, Tuskan et al. 2006) and the pathogen (*Melampsora larici-populina*, Duplessis et al. 2011) genome sequences. Populations of this fungal pathogen have been deeply impacted by host domestication (Xhaard et al. 2011). In particular, the massive deployment of poplar resistance 7 imposed a strong selection pressure and caused an extensive selective sweep in pathogen populations after resistance breakdown. Here we aim to benefit from historical samples (dating back to before the resistance breakdown which occurred in 1994) to unravel genome evolution of this pathogenic fungus during this major event of selection and subsequent population expansion. We combined population genetic data from neutral (25 microsatellite markers) and selected markers (10 small secreted protein sequences) plus the genome sequences of 12 additional individuals collected at different times. First we deciphered the population structure and demographic history of this pathogen following the breakdown of poplar resistance 7 from neutral genetic data. Second we detected the genomic regions hitchhiked by this selection event from the genome scan analysis of the 12 individuals and thanks to coalescent-based modelling (De Mita et al. 2007). Finally, we discuss the power of this method in identifying the gene(s) putatively responsible for the gene-for-gene interaction between rust and poplar.

Keywords: resistance breakdown, demographic history, genome scan, selective sweep

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When Resistance genes introgressed in agrosystems act as gateways for virulent populations from the wild: the apple scab case study**Leroy Th.¹; Lemaire Ch.¹; Gladioux P.^{1,2}; de Gracia M.¹; Guérin F.^{1,3}; Durel Ch. E.¹; Michaleka M.⁴; Caffier V.¹; Le Cam B.¹**

¹INRA, UMR1345, Institut de Recherche en Horticulture et Semences (INRA, Agrocampus-Ouest, Université d'Angers), SFR 4207 QUASAV, F-49071 Beaucouzé, France

²Plant and Microbial Biology, 321 Koshland Hall, University of California, Berkeley, CA 94720-3201

³Université de la Réunion, F-97715 Saint-Denis, France

⁴RIPF 96-100 Skierniewice, Poland

Abstract

Overcoming of a resistance gene is often described as a consequence of the spreading of a mutant that invades resistant hosts from infected susceptible cultivars ; invasion of virulent populations from the wild habitat being often neglected. Here, we intended to decipher the evolutionary history of the European populations of the apple scab pathogen *Venturia inaequalis* enable to overcome the *Rvi6* resistance gene introgressed in *Malus x domestica* from the crabapple *Malus floribunda*. We demonstrate (1) that virulence corresponding to a new resistance gene introgressed in agrosystems was previously present in the wild within a population that has diverged since several thousands years, (2) that deployment of the corresponding resistance gene in agrosystems can then act as gateways for virulent populations to invade orchards, (3) that secondary contact followed by mating between invading and resident population can reveal genetic incompatibilities (Dobzhansky-Müller Incompatibilities) accumulated during divergence between the two populations, (4) that resident populations can be replaced by a new population with modified genetic and pathogenicity traits. This study based on state-of-the-art tools of population genomics, phenotyping and genetic mapping is the first to demonstrate the occurrence of intrinsic post zygotic barriers in pathogens revealed by a secondary contact. Overall, it introduces the concept of the risk of generating new “hybrid” populations harbouring new pathogenic traits by introgression of resistance genes in agrosystems by breeders

Keywords: Emergence, virulence, secondary contact, *Venturia*, *Malus*, wild habitat, evolutionary history

Virulence patterns, genetic diversity and population structure of Australian *Bipolaris sorokiniana* isolates (teleomorph: *Cochliobolus sativus*) isolates

Horne M. ¹; Martin A. ¹; Platz G. ²; Sutherland M. ¹

¹Centre for Systems Biology, Faculty of Sciences, University of Southern Queensland Toowoomba, 4350, QLD, AUS

²Agri-Science Queensland, Department of Employment, Economic Development and Innovation, Hermitage Research Station, Warwick, Qld 4370, AUS

Abstract

Foliar spot blotch and common root rot are diseases which adversely affect the yield of wheat and barley worldwide. Both diseases are caused by the fungal pathogen *Bipolaris sorokiniana* (teleomorph: *Cochliobolus sativus*). Spot blotch (SB) in Australia is only of economic importance on barley in favourable seasons and occurs predominantly in QLD and northern NSW. Localised yield losses of up to 70% have been reported. Common root rot (CRR) occurs in most regions where wheat and barley are grown, and is most severe in water-stressed conditions, typically incurring losses of 10-15%. A study investigating the population diversity of 126 Australian *B. sorokiniana* isolates by cluster analysis of amplified fragment length polymorphisms (AFLP) in the genomic DNA has been completed. Isolates collected between 1995 and 2011 from various plant tissues were analysed including leaves, seeds, stubble tissue, roots and crown tissues. When subjected to cluster analysis, two populations were evident, one consisting mostly of isolates from roots, stubble and below-ground tissues. The other consisted entirely of isolates from leaves and seeds. The clustering of isolates suggests a level of tissue specificity of the pathogen. To further investigate the level of host and tissue specificity of various isolates of the pathogen and the degree of disease resistance in the host, the virulence of sixteen fungal isolates from different hosts and tissues are being tested on a differential set including ten barley and eight wheat genotypes of varied disease susceptibility.

Key words: *Bipolaris sorokiniana*, *Cochliobolus sativus*, barley, wheat, virulence

Session 2
Sustainable and integrated
breeding
and
deployment of genetic resistance

Broad-spectrum disease resistance in crop varieties for an unpredictable future

Brown, James K. M.

John Innes Centre, Colney, Norwich, NR4 7UH, England

Abstract

Sustainable, efficient food production requires adequate disease control in crops. While most research in plant pathology studies single species in controlled environments, crops are assailed by numerous parasites in variable conditions. Farmers require crop varieties which combine adequate resistance to all currently significant parasites with the more important traits of maximal yield and excellent quality. This challenge will be made even harder in future by accelerating climate change, insensitivity of parasites to environmentally-benign pesticides, and stringent regulation of chemicals in agriculture. Resistant varieties will therefore become even more vital to farming than they are already.

Plant breeding has been a cornerstone technology in global food security, not least in producing varieties resistant to disease. It uses an evolutionary approach which is essentially Darwinian selection, accelerated greatly. It is particularly successful in advancing many traits simultaneously, including disease resistance, when there is plenty of genetic diversity available, the breeding process is efficient, and selection nurseries make reliable predictions of the future performance of varieties on farms. A challenge for the future will be to continue making advances in yield despite a less predictable climate and a need for better disease-resistance. A significant complication is that there is increasing evidence that several types of disease resistance incur trade-offs with traits such as yield and resistance to other diseases.

Genetic diversity will be crucial in overcoming future threats to food production. An increasingly useful tool in breeding is association genetics, which analyses many varieties relevant to a plant breeding objective rather than a cross between two contrasting genotypes. The lower precision of association genetics compared to the conventional approach, given populations of similar size, is balanced by the opportunity to identify useful genes in diverse branches of the family tree of varieties. This can be exploited by making crosses from diverse lineages to achieve transgressive segregation, such that progeny lines have better resistance than either parent. Other advantages of the association approach are that it can locate useful genes in well-adapted germplasm, rather than exotic material; it can discover genes with a positive effect on one trait but minimal detriment to other traits; and an established resource of pedigree and marker data and seed stocks can be deployed rapidly to defend against a new threat to agriculture.

An over-arching approach for resistance breeding in arable crops in future may be to select for acceptable susceptibility to all current diseases while maintaining resources required to adapt crops to future challenges. The significance of resistance with respect to more important traits, yield and quality, must not be exaggerated in selection of new varieties, while a weak link, in the form of high susceptibility to one disease despite good resistance to others, threatens the feasibility of disease control in crops.

Examples will be drawn from *Septoria tritici* blotch of wheat, *Ramularia* leaf spot of barley and powdery mildew of cereals.

Keywords: over-arching approach; resistance breeding; cereals

Disease-suppressive landscapes – what role for spatial deployment of genetic resistance?

Walter A.H. Rossing¹, Jeroen C.J. Groot¹, Geert J.M. Kessel², Edith T. Lammerts van Bueren³, Wopke van der Werf⁴

¹ Wageningen University, Farming Systems Ecology, Wageningen, The Netherlands

² Plant Research International, Wageningen, The Netherlands

³ Wageningen University, Plant Breeding, Wageningen, The Netherlands

⁴ Wageningen University, Crop and Weed Ecology, Wageningen, The Netherlands

Abstract

There is general agreement that future agriculture needs to produce more, but with less inputs and less environmental impact. This paper explores to which extent spatial deployment of genetic resistance – captured by the intuitively appealing notion of disease-suppressive landscapes – may be expected to add to the existing toolkit of plant epidemiology, taking late blight of potato as an example.

We will first consider the bio-physical system and assess knowledge on pathogen and disease spread at different spatial scales. Spatially explicit modelling provides a powerful means of knowledge integration and yields quantitative information. Moving from whole plants to fields and to mosaics at the scale of square kilometres, the relative importance of different local management options on ecological connectivity of hosts can be considered. Results from our work at multiple spatial scales will be used to discuss the relative importance of initial spore load, fungicide use, landscape composition and configuration, and weather on late blight epidemics.

Moving from the disease triangle to the disease tetrahedron, we will address the role of decision making by farmers and breeding companies in resistance management. Preliminary analyses in the Netherlands suggest potential trade-offs between private and public benefits associated with spatial deployment of resistance. We will discuss the role of scientific knowledge for multi-level governance of host-pathogen interactions.

Keywords: disease-suppressive landscapes; plant epidemiology; late blight; potato

How effective is the durable resistance when encountering invasive strains? The case of wheat yellow rust pathogen *Puccinia striiformis*

Sørensen C. K.¹; Dedryver F.²; Leconte M.³; Hovmøllera M. S.¹; De Vallavieille-Pope C.³

¹ Aarhus University, Science and Technology, Department of Agroecology, DK4200 Slagelse, Denmark

² INRA UMR 118 Amélioration des Plantes et Biotechnologies Végétales, Le Rheu cedex, France

³ INRA, UR1290 BIOGER-CPP, BP 01, 78850 Thiverval-Grignon, France

A resistance is considered durable in a particular epidemiological environment a posteriori after being used at large scale for a long period of time. An important question is whether this type of resistance can withstand the invasion of new pathogen strains? Some French wheat cultivars combining genes for specific and partial resistance have remained effective against yellow (stripe) rust caused by *Puccinia striiformis* (Pst) for decades (Mallard et al. 2005; Dedryver et al. 2009). A set of recombinant inbred lines (RILs) has been developed to study the genetics of QTLs from these cultivars which includes cv. Camp Rémy, cv. Renan and cv. Apache. These cultivars have shown full resistance in France since the 1980s.

However, a new high temperature adapted aggressive strain of *Puccinia striiformis* (Pstst2) reached France in 2004 (Hovmøller et al. 2008). In other parts of the world this strain has caused severe epidemics and completely replaced old populations. Further, a new multivirulent genotype (pathotype 239E171V17) emerged in North Western Europe in 2011. The question is can partial resistance effective against old European Pst populations withstand the threat from these new invasive strains? In a greenhouse trial we assessed seedling and adult plant resistance of RILs from crosses between cv. Camp Rémy and the susceptible cv. Récital against the two new strains and one pathotype dominant in the old French Pst population. The RILs containing QTLs partially effective against the old pathotype were fully effective against the PstS2 strain, while they were intermediate to high susceptible to the multivirulent 239E strain. The cv. Récital was found to contain a previously undetected partial resistance against 239E and full resistance was seen in some lines combining QTLs from cv. Camp Rémy with this resistance in cv. Récital. The same RILs together with RILs from crosses of cv. Apache with cv. Taldor and of cv. Renan with cv. Récital were also tested against 239E and the old isolate in a field trial. This field trial revealed similar changes in the effectiveness of QTLs towards the old pathotype and new 239E strains as observed in the greenhouse. In conclusion our results clearly showed changes in the effectiveness of the components of durable resistance sources, while revealing the existence of resistance in cultivars previously considered susceptible. These results should encourage maintaining a high diversity of sources of resistance, not only from cultivars which already show resistance but also from susceptible parents which could provide resistance in the context of invasive strains.

Keywords : *Puccinia striiformis*, Wheat, Durable resistance, Pathogen evolution, Gene flow.

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Components of quantitative resistance to leaf rust in wheat cultivars

Azzimonti G.¹; Papaix J.¹; Marcel T.¹; Paillard S.²; Sache I.¹; Lannou C.¹; Goyeau H.¹

¹INRA, UMR1290 BIOGER-CPP F-78850, Thiverval-Grignon, France

²INRA, UMR 118 APBV, 35653 Le Rheu, France

Abstract

Based on assumptions of non-specific host-pathogen interactions, a complex genetic basis, and diversified underlying resistance mechanisms, quantitative plant resistance is generally expected to be durable. However, selection for quantitative traits can lead to increased pathogenicity, and to the erosion of a host quantitative resistance. Evaluating the likelihood for a pathogen to adapt to quantitative resistance requires i) to precisely measure the traits of the host-pathogen interaction, i.e. the resistance components, that are involved, and to investigate their specificity, ii) to establish their genetic basis and iii) to quantify their contributions in disease reduction at the scale of a field epidemic. Breeding for quantitative resistance could be based on combination of diversified resistance components. This could render the resistance more difficult to overcome by the pathogen, which would

have to adapt to different constraints at the same time.

Our study will contribute to this objective for wheat leaf rust, caused by *Puccinia triticina*.

Eight wheat genotypes were selected and confronted in a greenhouse to three isolates, belonging to different pathotypes. A high diversity and variability was expressed in the host x isolate combinations investigated for five components of resistance: infection efficiency (IE), latent period (LP), lesion size (LS), spore production per lesion (SPL), and spore production per unit of sporulating tissue (SPS). Isolate-specificity was found for all these components.

The genomics regions involved in resistance components of two of the cultivars were characterised in a doubled haploid population by QTL analysis. From the eight QTLs found, four were associated to the resistance expressed on a single component, and three of them to the resistance expressed on two components. All these QTLs were also associated to global disease reduction in field epidemic conditions.

In order to estimate the impact of the different components, measured in controlled conditions, on the resistance level in field conditions, disease severity (DS) was measured at three different times of epidemic development in the field. Statistical models were developed to provide the estimations of each trait for each cultivar-isolate pair, and correlation between traits was tested. All the components, except for SPL, participated to quantitative resistance in field epidemic conditions in different ways. The effect of LP in resistance was cumulative over the epidemic, whereas sporulation components effect (LS and SPS) was more important at the beginning of the epidemic, and decreased as the epidemic progress. IE had a more important effect on resistance in the middle of the epidemic.

Measurements of resistance components in controlled conditions allowed us to reveal phenotypical and genetical diversity in quantitative resistance to wheat leaf rust. Combining QTLs identified in this study, which were associated to different components, will diversify the mechanisms of resistance, taking into account the pathogen specificity, and protecting plants at the different stages of the epidemic. Altogether, this strategy should lower the risk of erosion of the available sources of quantitative resistance.

Keywords: Quantitative resistance, Wheat leaf rust, QTL analysis, Statistical modelisation

The durability of a major resistance gene is affected by quantitative trait loci which also confer quantitative resistance to virus.

Quenouille-Lederer J.^{1,2}; Paulhiac E.¹; Mistral P.¹; Némouchi G.¹ Sage-Palloix A.M.¹; Savio B.¹; Simon V.²; Moury B.²; Palloix A.¹

¹ INRA PACA, UR 1052 Génétique et Amélioration des Fruits et Légumes (GAFL).

² INRA PACA, UR 407 Pathologie Végétale.

Abstract

Genetic resistance provides efficient control of crop diseases but is limited by pathogen adaptation to resistant cultivars. The durability of the *pvr2³* allele, conferring resistance to *Potato virus Y* (PVY) in pepper (*Capsicum annuum*), was demonstrated to depend on the plant genetic background which modulates the frequency of *pvr2³* resistance breakdown due to virus mutation. Our objective was to identify, in this genetic background, quantitative trait loci (QTLs) controlling the breakdown frequency of the *pvr2³* major gene (resistance durability) and to compare them to the QTLs controlling quantitative resistance.

The QTL analysis was performed using a population of 310 doubled-haploid (DH) lines issued from the F1 hybrid between pepper line, 'Perennial' carrying *pvr2³* in a partially resistant background, and 'Yolo

Wonder' carrying the susceptible *pvr2*⁺ allele in a susceptible background. The DH lines were genotyped with 234 markers and the linkage map was established. The breakdown frequency of *pvr2*³ was assessed in the "*pvr2*³ sub-population" of 156 DH lines carrying the *pvr2*³ allele but segregating for the genetic background, after inoculation with a wild-type PVY cDNA clone avirulent (not infectious *per se*) towards *pvr2*³. The quantitative resistance (PVY accumulation and area under disease progress curve (AUDPC)) was assessed using the same PVY clone inoculated to the "*pvr2*⁺ sub-population" of 154 DH lines from the same progeny but carrying the *pvr2*⁺ susceptibility allele. The same quantitative resistance traits were also assessed in the "*pvr2*³ sub-population" inoculated by a mutant of the previous PVY clone carrying a single mutation allowing the breakdown of *pvr2*³.

Genotypic variance was highly significant for all traits with heritabilities of 0.87 for the breakdown frequency of *pvr2*³ and 0.47 to 0.62 for quantitative resistance traits. Two main QTLs, explaining 29% and 10% of the variance of *pvr2*³ breakdown frequency were identified on chromosomes 3 and 1. Considering the virus accumulation, 2 to 3 significant QTLs were detected, including the QTL on chromosome 3 which displayed a major effect on virus accumulation in the "*pvr2*³ sub-population" but a minor effect in the "*pvr2*⁺ sub-population", suggesting an interaction between this QTL and the alleles at the *pvr2* locus. For the AUDPC trait, 3 to 4 QTLs were significant including the QTLs on chromosomes 1 and 3 previously detected for resistance breakdown frequency.

Comparative mapping of the different traits showed that the two QTLs controlling the breakdown frequency of the *pvr2*³ allele are also involved in quantitative resistance, indicating that QTLs for quantitative resistance in the genetic background have a pleiotropic effect on the durability of the major resistance gene. Interestingly, the amplitude of the effect on virus accumulation of the QTL on the chromosome 3 depends on the allele at the *pvr2* locus, suggesting functional interaction between these loci. This first mapping of QTLs directly affecting resistance durability opens the way for sustainable resistance breeding.

Keywords: Resistance durability, quantitative resistance, resistance breakdown, Capsicum, Potato virus Y, major resistance gene, QTL analysis, eukaryotic translation initiation factor 4E.

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Experimental evidence of the efficiency of two resistance genes deployment strategies - pyramiding or alternating - for sustainable management of root-knot nematodes

Djian-Caporalino C.¹; Fazari A.¹; Marteu N.¹; Sage-Palloix A.M.²; Risso S.³; Lanza R.³; Palloix A.²; Castagnone-Sereno P.¹

¹INRA PACA (Provence Alpes Côte d'Azur), UMR1355 INRA/UNSA/CNRS, Institut Sophia Agrobiotech, BP167, F-06903 Sophia Antipolis, France

²INRA PACA, UR1052, Génétique et Amélioration des Fruits et Légumes, F-84143 Montfavet, France

³Chambre d'agriculture des Alpes Maritimes, 1 route de Grenoble, 06200 Nice, France

Abstract

The current restrictions of the use of chemical nematicides have contributed to increase root-knot nematode (RKN) problems in horticultural crops. In this context, plant resistance appears as the most effective and sustainable method of control. In horticultural crops, a few major resistance (*R*) genes are available, but the possible occurrence of virulent nematode populations able to reproduce on *R*-plants may constitute a severe threat to this control strategy. Here, we tested several *R*-gene deployment strategies in the pepper-nematode interaction to implement a rational management of the *R*-cultivars increasing the sustainable management of RKN. Experiments were conducted in climate-controlled rooms, in greenhouses, and under 3-years-field agronomic conditions comparing i) the succession of the same *R*-gene every year, when introgressed in a resistant vs. a susceptible genetic background, ii) the alternance of single *R*-genes in rotation, iii) the mixture of genotypes bearing single *R*-genes sown in the same plot, and iv) the pyramiding of two *R*-genes in one genotype. Results allow the identification of conditions lowering the emergence of virulent nematodes and assess the time required for the sustainable improvement of soil health (reduction of parasite populations under their damage threshold) using the *R*-plants as RKN "traps". Alternating different *R*-genes in rotation was confirmed to be efficient to decrease virulent populations in fields due to the specificity of the virulence previously demonstrated in laboratory experiments. Suppression of the emergence of virulent isolates because of the pyramiding of two different *R*-genes in one pepper genotype was confirmed in controlled and natural conditions and proved durable during a 3-years-field experiment. Recently, a new project called 'GEDUNEM' has been launched in the framework of the INRA metaprogramme SMaCH (Sustainable Management of Crop Health). It aims at identifying innovative strategies across the agrosystem, combining varietal resistances in crop rotations with agronomic practices, for the sustainable and integrated management of RKN in protected vegetable cropping systems.

Keywords: sustainable crop protection, breeding strategy, resistance gene deployment, virulence emergence, root knot nematodes, *Meloidogyne spp.*, *Capsicum spp.*

Sustainable control of apple scab obtained by the association of cultivar resistance, sanitation and soft chemical control

Didelot F.¹; Parisi L.²; Orain G.³; Lemarquand A.³; Caffier V.¹

¹INRA, UMR1345, Institut de Recherche en Horticulture et Semences (INRA/AgroCampus-Ouest/Université d'Angers), SFR 4207 QUASAV, F-49071 Beaucouzé, France

²INRA-UERI – Domaine de Gotheron – 26320 SAINT-MARCEL-LES-VALENCE, France

³INRA Centre d'Angers, UE horticole, F-49071 Beaucouzé, France

Abstract

The resistance of cultivars can be qualitative and conditioned by single gene or quantitative and conditioned by multiple genes of partial effect, with a 'great deal of gray area' between these two extreme categories. Qualitative resistance has a high efficiency but generally risks to be overcome by virulent isolates of the pathogen, whereas quantitative resistance is generally considered as more sustainable but may be insufficient for an efficient control of the disease. In both cases, it is therefore necessary to develop strategies that integrate resistant cultivars with other methods for a better efficiency and sustainability of the disease control. Our aim was to test a strategy that integrates apple resistant cultivars with sanitation (removing of foliar litter) and reduced number of fungicide sprayings (applied only in case of important risks of contamination) for the control of scab. We studied two different cultivars 'Reine des Reinettes' and 'Ariane'. 'Reine des Reinettes' is a partially resistant cultivar, grown for a long time with no indication of resistance erosion overtime; but its resistance is not sufficient when weather conditions are very favourable to scab. 'Ariane' carries the major resistance gene *Rvi6* (=Vf), which is overcome by virulent isolates of *Venturia inaequalis*. In our experimental orchard, situated in Loire valley, the frequency of virulent isolates to *Rvi6* was very low at the beginning of the experiment. Our results showed (1) a stability of the efficiency of scab control on 'Reine des Reinettes' (less than 2% of scabbed fruits at harvest) over a six-year period, despite weather conditions that were very favourable to scab (until 85 % of scabbed fruits on the plot without sanitation and fungicide treatments); (2) a delay of two years in the breakdown of 'Ariane' resistance (increase from less than 2% to more than 20% of scabbed fruits) compared to the modality without sanitation and fungicide treatments, despite conditions more favourable to the development of the disease than in commercial orchards. These results showed the potential of a strategy that integrate resistant cultivars with other methods for a sustainable control of the disease with a reduction of 50% of the number of sprayings compared to a susceptible cultivar managed in Fruit Integrated Pest Management in the same weather conditions. Such a strategy may be not sufficient to avoid the breakdown of a cultivar with a major gene on the long term, but is very promising in case of partial resistance, even in the case where this resistance may be eroded due to the adaptation of pathogen populations.

Keywords: Partial resistance, apple scab, methods of disease control, sanitation

Integrating the building blocks of agronomy and biocontrol into an IPM strategy for wheat stem sawfly

Beres B. L.^{1, 2}; Cárcamo H. A.²; Weaver D.K.³; Dosdall L.M.¹; Evenden M.L.⁴; Hill B.D.²; McKenzie R. H.⁵; Cai Yang R.¹; Spaner D. M.¹

¹U. of Alberta, Edmonton, Alberta, AB

²Agriculture and Agri-Food Canada, Lethbridge, AB

³Montana State University, Bozeman, MT

⁴University of Alberta, Edmonton, AB

⁵Alberta Agriculture & Rural Development, Lethbridge, AB

Abstract

The wheat stem sawfly (*Cephus cinctus* Norton [Hymenoptera: Cephidae]) is a serious threat to wheat (*Triticum aestivum* L.) and other cereal grains in the northern Great Plains. Insecticides have proven ineffective for sawfly control and can be detrimental to beneficial insects. The management of wheat stem sawfly, therefore, requires the integration of host plant resistance, agronomic and biological control strategies. Recent studies in Alberta, Canada have assessed the response of wheat stem sawfly and its natural enemies to cultivar selection, residue management, seeding rates, fertility regimes, and harvest management. Solid-stemmed cultivars are usually agronomically superior to susceptible cultivars when sawflies are present. The stubble disturbance associated with residue management and direct-seeding in a continuous cropping system can reduce sawfly populations compared to a wheat-fallow system. Increased seeding rates can optimize yield, but an inverse, negative relationship between pith expression (stem solidness) and higher seeding rates may occur. Positive yield responses are typically observed with N rates > 30 kg N ha⁻¹, but increased insect stem cutting by sawfly can occur with higher N rates. Increasing cutter bar heights during combine harvest can conserve natural enemies, and chopping straw for improved residue management in the spring will not likely affect wheat stem sawfly parasitoids that overwinter in the straw. In summary, an integrated strategy to manage wheat stem sawfly consists of diligent pest surveillance, planting solid-stemmed cultivars, continuous cropping with appropriate pre-seed residue management, seeding rates no greater than 300 seeds m⁻², 30 to 60 kg N ha⁻¹, and harvest cutting heights of at least 15 cm to conserve parasitoids.

Keywords: IPM strategy; wheat stem sawfly; *Triticum aestivum*

Exploring the potential of landscape diversification for limiting epidemic risk

Lannou C.¹; Papaix J.^{1,2}; Goyeau H.¹; David O.²; Touzeau S.²; Monod H.²

¹INRA, UR 1290 BIOGER, 78850 Thiverval Grignon, France

²INRA, UR 341 Mathématiques et Informatique Appliquées, 78350 Jouy-en-Josas, France

Abstract

The interest of host diversification for limiting epidemic development has mainly been explored at the scale of individual fields. Theoretical and experimental studies have shown that mixing varieties bearing different resistance genes in a field creates a more resistant host population and hampers the development of epidemics. The idea of extending this approach to larger spatial scales has been raised more than 20 years ago (Priestley & Bayles 1980, Mundt & Brophy 1988) but has not met so far much interest in plant pathology. Nowadays, the drastic restriction in the use of pesticides in agriculture, resulting either from new EU regulations on product registration or from governmental policies for a healthier environment renews the interest for large-scale approaches for disease control.

In the past four years, we investigated the potential of landscape diversification for reducing the disease risk in major crops. For this, we developed a landscape epidemiology approach based both on data analysis and modeling. Our work was largely based on the *Puccinia triticina* – wheat pathosystem but we attempted to keep the theoretical approaches as generic as possible. We present here the main results.

A combined analysis of three datasets (the frequency of the main pathotypes in the *P. triticina* French population, the frequency of the main varieties grown by farmers, and the observed disease severity on those varieties throughout the country) established a link between the observed resistance level of a variety and the landscape varietal composition at the national scale. We showed that the pathotype frequencies on a variety were influenced by the frequency of that variety in the landscape but also by the frequency of the other varieties.

We developed a modeling framework including an epidemic model and procedures for generating landscapes with different structures and for managing the pathogen dispersal on a spatially explicit domain. We first explored the dynamics of the pathogen population on landscapes with more or less aggregated variety distributions. We found that the overall disease severity was generally lower when the resistant and susceptible fields were mixed but, under some conditions, aggregating the resistant fields was more efficient. This depended on the quantitative level of resistance, on the frequency of the varieties and on the dispersal capacity of the pathogen.

In a second step, we explored the pathogen evolution in the landscape under different conditions of host distribution. We showed that host heterogeneity influences the global evolutionary trajectory of the pathogen. In particular, grouping a variety in the landscape tends to favor the adaptation of pathogens to this variety. On the contrary, spatially diversified landscapes seem to favor generalist pathogens.

This work is a first approach but suggests that considering host diversification at the scale of a production area is promising and could lead to new possibilities for the sustainable management of resistant varieties. From these results, different perspectives could be considered, either from an epidemiological point of view or in terms of acceptability and practical implementation.

Keywords: resistance diversification, landscape epidemiology, modeling

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Durable plant disease resistance by evolution management - how?

Lof M. E.¹; van der Werf W.²

¹Centre for crop system analysis, Wageningen University, Wageningen, The Netherlands

²Centre for crop system analysis, Wageningen University, Wageningen, The Netherlands

Abstract

Durable resistance to plant pathogens is highly desired but hard to achieve. In a modelling study, we explore the factors that promote or jeopardize durability of resistance. The purpose is to derive strategies of host plant breeding and spatio-temporal plant genotype deployment that reduce as much as possible the rate of resistance breaking. In other words: the purpose is to manage evolution. The model is multi-scale and long term, and the spatial extent is a continent. The host and pathogen populations are modelled as meta-populations with gene for gene relationships governing compatibility.

Pathogen genetics is modelled as a population process of different genotypes, with multiplication, survival, dispersal, mutation and selection as key processes. The time step is a generation of the pathogen, the run time centuries. The distribution of different genotypes of the host is modelled on the basis of scenarios for genotype deployment by farmers and strategies for breeding new varieties in response to breaking of resistance at field level. Strategies for evolution management include the stacking of genes, rotation of plant resistance genotypes, fine or broad scale mixing of varieties, etc. What are the pros and cons of different strategies?

The model will be applied to pathosystems of global importance, particularly yellow rust of wheat (*Triticum aestivum*), caused by the fungal pathogen *Puccinia striiformis* f. sp. *tritici* and Septoria leaf blotch, caused by *Mycosphaerella graminicola*. For both diseases, the development of durable resistance is of great importance. Model parameterization will be achieved by a combination of analysis of process-based information on pathogen life tables and analysis of historical patterns of pathotype dynamics (inverse modelling). The modelling results will be linked to field work in the PURE project that aims at the development of European farming systems that reduce the use of pesticides (<http://www.pure-ipm.eu/project>).

Keywords: durable resistance, resistance gene deployment, metapopulation model, mutation and selection

Sustainable plant resistance management in agricultural landscapes

Fabre F.¹; Rousseau E.^{1,2}; Mailleret L.^{2,3}; Moury B.¹

¹INRA, UR 407 Unité De Pathologie Végétale, F-84140 Montfavet, France

²INRA, UR 880 URIH, 400 route des Chappes, BP 167, F-06903 Sophia Antipolis, France

³INRIA, Biocore Team, F-06902 Sophia Antipolis, France

Abstract

The deployment of resistant crops often leads to the emergence of resistance-breaking pathogens that suppress the yield benefit provided by the resistance. The theoretical analyses presented are designed to provide guidelines for farmers aiming altogether to optimise the deployment of a resistant cultivar in a landscape over several years. We explored how farmer main leverages (resistant cultivar choice, resistance deployment strategy, landscape planning, cultural practices) can be best combined in a given epidemiological context (defined in a fully susceptible landscape by the epidemic intensity and the landscape structure) to achieve resistance durability while minimising yield losses due to plant viruses.

Assuming a gene-for-gene type of interaction, virus epidemics are modelled in a landscape composed of a mosaic of resistant and susceptible fields, subjected to seasonality, and of a reservoir hosting viruses year round. The model links the genetic and the epidemiological processes shaping at nested scales the demo-genetics dynamics of viruses. The description of the seasonality leads us to use a semi-discrete modelling approach that is a hybrid dynamical system that undergoes continuous dynamics in ordinary differential equations most of the time (describing describes the in-season epidemic dynamics in fields) and that experiences discrete dynamics (mimicking pathogen overwintering in reservoirs) at some given time instants.

The choice of the resistance gene (characterized by the equilibrium frequency of the resistance-breaking virus at mutation-selection balance in a susceptible plant) is the most influential leverage of action. In landscapes with low to intermediate epidemic intensities (*i.e.* when the mean proportion of plants infected during a season is lower than 0.5 before resistance deployment), resistance genes defeated by the accumulation of two mutations in the viral genome) are likely to be durable, whatever the proportion of resistance released. The deployment of such resistance genes significantly reduces the

overall damage caused by the pathogen. Depending on the landscape structure considered (*i.e.* on the connectivity between the fields and the reservoir hosts), this reduction can be more than proportional to the proportion of resistance released. By contrast, resistance genes defeated by a single mutation can only be durable in landscapes with low epidemic intensities (*i.e.* when the mean proportion of plants infected during a season is lower than 0.1).

Our results also showed that optimal of resistance deployment (*i.e.* strategies maximizing the yield increase provided by resistance deployment) range from mixture (where susceptible and resistant cultivars coexist) to pure strategies (with only resistant cultivar) depending on the resistance characteristics and on the epidemiological context (epidemic incidence, landscape connectivity). For example, by releasing the resistance at high cropping ratios in landscapes where epidemics are primarily driven by transmission from the reservoir hosts (*i.e.* situation of pathogen spillover), it is almost possible to suppress the virus from the reservoir.

Finally, we demonstrate and discuss gaps concerning virus epidemiology across the agro-ecological interface that must be filled to achieve sustainable disease management.

Keywords: Deployment strategy; Durable resistance; Evolutionary epidemiology; Gene-for-gene model; Landscape epidemiology.

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Plant resistance deployment to control a diversified pathogen population: apple scab as a modelling case study

Camara B.I. ^{1,2}; Caffier V. ¹; Didelot F. ¹; Le Cam B. ¹; Sapoukhina N. ¹

¹IRHS (INRA, Université d'Angers, Agrocampus Ouest), SFR QUASAV, rue G. Morel F-49071 Beaucouzé, France

²Laboratoire LIEBE UMR CNRS 7146, Université Paul Verlaine-Metz, av. du Général Delestraint, 57070 Metz, France

Abstract

The spatial and genetic heterogeneity imposed by a host diversification strategy alters both specialization of pathogen pathotypes and competitive interactions among them. We studied the effects of pathogen specialization and differences in pathotypes_' infection efficiencies on the outcomes of a host diversification strategy. We constructed a reaction-diffusion model describing the dynamics of a diversified host-pathogen system in a two-dimensional spatial domain. Our work is of a generic nature and applies to a wide range of diseases with diffusive pattern of dispersal, but we used apple scab disease, caused by *Venturia inaequalis*, as an example. We parameterized the model, using field data for the spread of this typical airborne fungal disease over a heterogeneous orchard including susceptible and resistant varieties. With the parameterized model we demonstrated that the choice of the components of diversification strategies should be consistent with differences in pathotypes_' infection efficiencies. Moreover, consideration of among-pathotypes interactions made it possible to highlight the key-principles of the sustainable use of quantitative resistance. Our theoretical findings might also contribute to clarifying the usefulness of_'low selective pressure_' strategies, such as low pressure drugs or chemicals and imperfect vaccines, in the management of drug- and chemical-resistance.

Keywords: reaction-diffusion model, plant resistance management, quantitative resistance, competition, heterogeneity, Bayesian method

Session 3
From plant-pathogen
molecular interactions
to the durability of resistance

Molecular co-evolution between pathogens and wild tomatoes

Rose L.

Institute of Population Genetics, Heinrich-Heine-Universität

Abstract

Resistance genes typically show evidence of rapid protein evolution in natural host populations. Variability at these loci may be the direct result of a co-evolutionary arms race between host and pathogen. However, the abundance of pseudogenes also segregating in natural populations at well-known resistance genes indicates that the strength of natural selection likely fluctuates and these genes may be subject to bouts of relaxed constraint as well.

In my lab, we investigate the strength and target of natural selection within resistance genes present in natural host plant populations of *Arabidopsis* and wild tomatoes. Using a combination of population genetic studies of well-characterized pathogen resistance genes and functional studies involving protein expression, enzymatic assays, plant transformation and pathogen infection studies, we have determined how naturally occurring protein variation in host populations affects defense to pathogens at the individual level. I will describe the results from these studies and discuss how we can apply what we have learned about natural selection in the wild to discover ways to improve the durability of resistance in our crop species.

Keywords: natural selection resistance genes, *Arabidopsis*, tomatoes

Constraints on resistance and pathogenicity factors and their consequences on resistance durability

Moury B.¹; Caranta C.²; Ewert S.²; Lacombe S.²; Fabre F.¹; Palloix A.²

¹INRA, UR407 Pathologie Végétale, F-84140 Montfavet, France

²INRA, UR1052 Génétique et Amélioration des Fruits et Légumes (GAFL), F-84140 Montfavet, France

Abstract

Most recessive resistances to potyviruses in solanaceous crops are controlled by eukaryotic translation initiation factors 4E (*eIF4Es*). Physical interaction between *eIF4E* and the viral protein genome-linked (*VPg*) allows potyviruses to infect the plant. Amino acid substitutions in *eIF4E* can disrupt the molecular interaction with *VPg*, which confers resistance to the plant. Similarly, amino acid substitutions in the *VPg* can restore interaction with the mutated *eIF4E* and confer resistance-breaking properties to the virus. Both *eIF4Es* and *VPgs* are highly constrained proteins and these constraints have important consequences on resistance specificity, resistance durability and the potential efficiency of management strategies.

Keywords: *Solanaceae*, *Potato virus Y*, *Potyvirus*, resistance breakdown

Analysis of the wheat *Lr34_Yr18* gene and the molecular basis of durable resistance Keller B.¹; Krattinger S. G.^{1,2}; Selter L. L.¹; Risk J. M.²; Harsh Ch.¹; Lagudah E. S.²

¹Institute of Plant Biology, University of Zurich, Zollikerstrasse 107, 8008 Zurich, Switzerland

²CSIRO Plant Industry, GPO Box 1600 Canberra, ACT 2601, Australia

Abstract

The wheat *Lr34/Yr18* gene has been used in agriculture for more than 100 years. In contrast to many other resistance sources against leaf and stripe rust, it has remained effective and no pathogen adaptation has ever been reported. This makes *Lr34* a unique and highly valuable resource for rust resistance breeding. The *Lr34/Yr18* gene acts pleiotropically with the following activities: it confers partial resistance to the rust pathogen species leaf, stem and stripe rust, to powdery mildew as well as to barley yellow dwarf virus, and finally, it induces leaf tip necrosis in the absence of any pathogen infection. The durability of this gene suggests a molecular mechanism of resistance which is different from major gene resistance. This is supported by the molecular nature of *Lr34* which was found to encode an ABC transporter (Krattinger et al. 2009). Interestingly, all tested wheat lines contain an allele of the *Lr34* gene on chromosome 7DS. In its susceptible form, the gene does not confer resistance. The difference between the encoded resistant and susceptible LR34 isoforms only consists of two amino acid changes, while the rest of the protein is identical. Susceptible forms of *Lr34* were also found in rice and sorghum (Krattinger et al. 2011). The two amino acid changes must change the biochemical properties of the resistant LR34 transporter in such a way that the plant develops resistance. We speculate that there is a slight conformational change in the resistant form of the protein, resulting either in modified specificity or kinetics of the transported molecule, or that the binding properties to an unknown second protein interacting with LR34 are changed, resulting in altered function. While the molecular nature of the molecule(s) transported by the LR34 protein remains unclear, it is likely that a physiological change related to *Lr34* activity is at the basis of resistance. We have made transgenic wheat lines with the *Lr34* resistance gene and found interesting variability of gene action possibly useful for breeding (Risk et al. 2012). We are currently establishing transgenic approaches in heterologous grass species to further investigate the molecular activity of *Lr34* and to better understand the physiological mechanisms resulting in disease resistance.

Keywords: ABC transporter, durable resistance, leaf rust, leaf tip necrosis, multi-pathogen resistance

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Molecular evolution of the *AvrLm7* avirulence gene of *Leptosphaeria maculans* under resistance gene selection in the field is driven by its genomic location, sexual reproduction and cropping practices

Balesdent M.H.¹; Daverdin G.¹; Gout L.²; Aubertot J.N.³; Pinochet X.⁴; Rouxel T.¹

¹INRA, UR1290-Biogère CPP, 78850 Thiverval-Grignon, France

²AgroParisTech, Grignon, 78850 Thiverval-Grignon, France

³INRA, UMR 1248 AGIR, BP 52627, 31326 Castanet Tolosan Cedex France

⁴CETIOM, 78850 Thiverval-Grignon, France

Abstract

Leptosphaeria maculans, the agent of stem canker of oilseed rape, develops “gene-for-gene” interactions with its host plant, and shows a high evolutionary potential to “breakdown” novel resistance sources in the field. The newly marketed *Rlm7* resistance allowed us (i) to monitor the evolution of populations faced to this resistance over three consecutive years, (ii) to identify the nature and sequence of molecular events responsible for the loss of the corresponding *AvrLm7* avirulence, and (iii) to compare the durability of *Rlm7* in two contrasted cropping systems, i.e. a “cautious” system involving a diversified crop sequence and ploughing of oilseed rape stubble after harvest, and a “risky” system without crop sequence and with simplified tillage. Population genetics approaches were used to evaluate the main population traits linked with birth and dissemination of the virulent mutations in the populations. A total of 1967 isolates were collected, over the three years, either on a cv. harbouring *Rlm7* or on a cv. devoid of it, then phenotyped and genotyped. During this period the ratio of *avrLm7* virulent isolates in local populations, as estimated from isolates collected on the susceptible cv., increased from 0 [0.0, 1.9] % to 36.2 [30.0, 42.7] % in the field submitted to the “risky” system. All possible molecular events leading to virulence were found within our sampling with an over-representation of complete deletions and wide-scale RIP mutations of the coding sequence. In contrast, less than 1% of *avrLm7* isolates were collected on the susceptible cv. in the field submitted to the “cautious” system. The high mutability of *AvrLm7* appeared to be mainly driven by its repeat-rich genome environment combined with the obligatory annual sexual stage that promotes large-scale deletions, translocations, duplications and RIP events, rather than gene flow, which appears to be a minor threat for the resistance breakdown in the *L. maculans*-*B. napus* pathosystem. The major role of the sexual stage in generating virulent isolates may explain the increased durability of *Rlm7* in the “cautious” system where ploughing prevents mating by burying stem residues on which pseudothecia and ascospores are produced. This study permitted to quantify the role of cropping practices in the preservation of the efficacy of a new specific resistance. This information can now be used to develop models aimed at helping the design of durable management strategies. At the national level, the frequency of *avrLm7* isolates in 2010-2011 still remains at a low level (less than 4%) in spite of 5 years of commercialization of *Rlm7* and its deployment on around 20% of oilseed rape acreage since 2008. The reasons for this limited expansion of virulent isolates will be discussed.

Keywords: molecular evolution; resistance breakdown; minimum tillage; mutation; phoma

Characterization of adaptation to a resistance gene with a pleiotropic effect, the *Vat* gene in melon.

Boissot N.; Thomas S.; Chareyron V.; Mistral P.

INRA, UR1052, Génétique et Amélioration des Fruits et Légumes, B.P. 94, F 84143 Montfavet cedex

Abstract

The deployment of resistance genes against aphids, is restricted because few genes have been characterised. The *Vat* gene, conferring resistance to *Aphis gossypii*, has been effectively deployed in melon crops since 20 years in South-eastern France. We have studied the adaptation of *A. gossypii* populations to that *Vat* gene.

Seven clones of *A. gossypii* were collected in melon producing areas where the *Vat* gene has been or not deployed. These clones were characterized using 8 SSR markers. The phenotypic variability of *A. gossypii* was shown matching the genetic variability revealed by eight microsatellite markers (Thomas et al, 2012). We phenotyped the seven clones using three resistant melon lines. One line was susceptible to all clones, the two other lines, Margot and 90625, were known to carry different alleles at the *Vat* locus. We took advantage of the double phenotype mediated by the *Vat* gene, the resistance to *A. gossypii* and the resistance to viruses (CMV, ZYMV...) when inoculated by *A. gossypii* – melon plants are susceptible to viruses when they are mechanically inoculated or inoculated by other aphids species.

Aphid clones triggered three phenotypic responses on Margot or 90625 lines: resistance to aphids and to the virus when inoculated by aphids, susceptibility for both phenotypes, and susceptibility to aphids and resistance to the virus when inoculated by aphids; we did not characterize any clones revealing a melon line resistant to aphids and susceptible to virus when inoculated by aphids.

Our results revealed a specific interaction between plant resistance alleles in melon and aphid clones. The *Vat* gene belongs to the CC-NBS-LRR resistance gene (Pauquet et al 2004). The avirulence factor in aphid, expected to interact with the *Vat* protein, is presently unknown, it should be excreted in saliva and introduced in plant tissue during puncturing, and then interacts with the *Vat* product triggering defence responses (Dogimont et al 2010). These responses are expected to be efficient against aphids and viruses. Our results suggested two adaptation mechanisms of aphids to the *Vat* mediated resistance: i/ suppression of both phenotypes, i.e. susceptibility to aphids and to virus when inoculated by *A. gossypii*, what suggests a suppression of the recognition step (non-adapted avirulence product), ii/ susceptibility to aphids associated with resistance to virus when inoculated by aphids, suggested that the recognition step occurs, triggering the defence responses that are efficient against virus but inefficient against aphids, i.e. aphids are adapted to these defence responses. We only observed adaptations of type i/ when considering the melon line 90625. Because the allele of resistance originating from this line has never been deployed in crops in areas we collected clones, these adaptations, observed in several clones, corresponded to an incomplete spectrum of the resistance efficiency. In South-eastern France, the resistance that has been deployed is mediated by the allele at the *Vat* locus present in Margot. In this area, where *A. gossypii* diversity is very large (Thomas et al 2012b), two clones, never observed before 2009, exhibited adaptation of type ii/ to that allele of resistance.

Keywords: NBS-LRR gene, aphid resistance, virus resistance

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Mutations in the Cylindrical Inclusion of Lettuce mosaic virus are associated with evolution towards resistance-breaking of eIF4E-mediated resistance in lettuce

Sorel M.; Svanella-Dumas L.; Acelin G.; Houvenaghel M.C.; Le Gall O.; Candresse T.; German-Retana S.

Equipe de Virologie, UMR BFP 1332, INRA Université de Bordeaux, BP 81, F-33883 Villenave d'Ornon, France.

Abstract

The Potyvirus genus is the largest genus of plant viruses and infects a broad range of hosts. It includes *Lettuce mosaic virus* (LMV), a major pathogen of worldwide distribution in commercial lettuce crops. In recent years, components of the eukaryotic translation initiation complex were identified as essential determinants in the outcome of RNA virus infection, in particular in the case of potyviruses. In particular, recessive allelic lettuce genes *mo1¹* and *mo1²*, currently used to protect lettuce crops against LMV, were shown to correspond to mutant alleles of the gene encoding the translation initiation factor eIF4E (German-Retana *et al.*, 2008; Nicaise *et al.*, 2003).

We have shown that the LMV resistance-breaking determinants map not only to the VPg encoding region (the main potyvirus eIF4E virulence determinant) but also to the C-terminus of the CI (Cylindrical Inclusion) helicase, providing the first example of a potyviral CI acting as a determinant for eIF4E-mediated resistance breaking (Abdul-Razzak *et al.*, 2009).

During a global survey of the biological and molecular diversity of LMV isolates, we showed that propagation of several non-lettuce isolates of LMV in *mo1¹* plants is accompanied by a gain of virulence correlated with the accumulation of mutations in the CI C-terminus, while the same isolates were stable when propagated in susceptible lettuce. A similar propensity to evolve towards resistance breaking was observed for synthetic chimeras between two parental LMV cDNA clones (LMV-0 avirulent and LMV-E virulent). Indeed, the ability of LMV-0 recombinants with a chimeric CI to overcome the *mo1* resistance was accompanied by the accumulation of mutations in the CI C-terminus, including the positions identified in the non-lettuce isolates after virulence acquisition. The contribution of each of the four identified mutations to resistance breakdown, their impact on the fitness of the virus and their epistatic relationships were further evaluated and the results obtained will be discussed in the context of the durability of *mo1* resistance.

Keywords: Recessive resistance breaking, eIF4E, lettuce, LMV, potyvirus, VPg, Cylindrical Inclusion

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Durable soybean cyst nematode resistance due to multiple copies of a multi-gene block at *Rhg1*

Bent A.¹; Cook D.¹; Guo X.^{1,3}; Lee T.G.²; Wang B.¹; Melito S.^{1,3}; Bayless A.¹; Hughes T.^{1,3}; Wang J.^{1,3}; Kim M.^{2,3}; Diers B. W.²; Jiang J.¹; Hudson M.²

¹University of Wisconsin - Madison, Madison, WI USA

²University of Illinois at Urbana-Champaign, Urbana, IL USA

³(authors with new current addresses)

Abstract

Soybean cyst nematode (SCN) is the most economically damaging pathogen of soybean (*Glycine max*) in the United States, causing over US \$1 billion in losses to growers annually. The soybean *Rhg1* locus is a large-effect QTL that, together with crop rotation, is the primary means of SCN disease control. Because of the expense and difficulty of assessing the SCN resistance phenotype, *Rhg1* is one of the most widely targeted loci for marker-assisted selection in any crop worldwide. Roughly 95% of the commercially cultivated soybean varieties marketed as SCN-resistant in the central U.S. utilize a single allele of *Rhg1*, the *rhg1-b* haplotype originally derived from *G. max* PI88788. Although SCN population shifts that partially reduce the efficacy of *rhg1-b* have been reported in some locations, this haplotype has been in widespread use for over 20 years and remains durable. The molecular basis of this SCN-resistance was not understood and there are no confirmed reports of a cloned SCN resistance gene. We previously defined a genetic interval for *rhg1-b* that, in the SCN-susceptible but fully sequenced Williams 82 variety, corresponds to a 67 kb genome segment carrying 11 predicted genes. In our recent work, silencing of genes in the *rhg1-b* genetic interval identified multiple tightly linked genes that each contribute to resistance in transgenic roots. The genes encode an amino acid transporter, an α -SNAP protein, and a protein with no recognized functional domains. DNA sequence, PCR and fiber-FISH analyses revealed a highly unusual locus structure: ten direct repeats of a 31.2 kb block that encodes these genes, with only a single copy in SCN-susceptible haplotypes. Transgenic overexpression of the single genes in SCN-susceptible roots was ineffective, but preliminary results indicate that overexpression of all genes from the 31.2 kb block confers enhanced resistance to SCN. Expression polymorphism attained by copy number polymorphism, rather than amino acid polymorphism, is apparently the primary cause of *Rhg1*-mediated SCN resistance. This unique combination of resistance-conferring features, and the extended durability of this resistance, will be discussed.

Keywords: Durable resistance, copy number variation

Loss-of-susceptibility as an alternative for pathogen resistance

Rodiuc N.^{1,2,3}; Barlet X.⁴; Perfus-Barbeoch L.^{1,2,3}; Marcel F.⁵; Quentin M.^{1,2,3};
Panabières F.^{1,2,3}; Abad P.^{1,2,3}; Bendahmane A.⁵; Marco Y.⁴; Keller H.^{1,2,3*}; Favery B.^{1,2,3*}

¹INRA UMR 1355 Institut Sophia Agrobiotech, F-06903 Sophia Antipolis, France.

²CNRS UMR 7254 Institut Sophia Agrobiotech, F-06903 Sophia Antipolis, France.

³ Université Nice-Sophia Antipolis, UMR Institut Sophia Agrobiotech, F-06903 Sophia Antipolis, France.

⁴Laboratoire des Interactions Plantes-Microorganismes, UMR CNRS 2594 - INRA 441, F-31326 Castanet Tolosan, France.

⁵URGV Génomique Végétale Versailles-Grignon, 2 rue Gaston Crémieux F-91057 Evry, France.

*equal contribution

Abstract

Biotrophic plant pathogens frequently trigger the differentiation of host cells into specialised feeding cells essential for successful infection. Oomycetes, fungi, and nematodes evolved the ability to exploit plant genomes and hijack host functions for their own requirements. Many approaches have been pursued to use *R* genes with the aim to engineer durable resistance to pathogens in plants. An alternative to achieve resistance is to exploit disease susceptibility i.e. plant genes that are required for disease. A loss of function of such genes might then lead to a recessive form of resistance. We found that oomycete and nematode infections were characterised by the tissue-specific transcriptional regulation of *Arabidopsis thaliana* *PSK Receptor 1* (*PSKR1*). Phytosulfokines (PSKs) are secreted sulphated peptide hormones widespread among plants. They act as mitogens, and their binding to the receptor triggers cellular de- and redifferentiation. *PSKR1* knockout mutants displayed a major impairment of feeding structure development and of oomycete and nematode reproduction, and were also less susceptible to a soil-borne bacterial pathogen. By contrast, all pathogens proliferated more efficiently in plants overexpressing the receptor or overproducing PSK. *PSKR1*-dependent disease developed independently of plant immune responses, instead reflecting the recruitment of host PSK signalling by pathogens to interfere with the cellular differentiation processes promoting infection. To transfer these findings to crops, we identified the *PSKR1* gene ortholog in tomato. We are presently characterising individual *PSKR1* mutants that we identified from TILLING populations. Because *PSKR1* is required for the establishment of the infection process, we predict that its inactivation in tomato results in a broad-spectrum disease resistance trait that is difficult to be overcome by pathogens. Furthermore, loss-of-susceptibility does not involve cost-intensive defense activation, and we thus expect that this resistance trait will not interfere with crop productivity.

Keywords: Susceptibility, feeding structures, nematode, oomycete, soilborne bacteria

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A mutation in an homologous of CPR5 is associated to resistance to Rice yellow mottle virus in rice

Albar L.¹; Bouniol J.¹; Thiemele D.^{1,2}; Kolade F.²; Chéron S.¹; Ghesquière A.¹

¹UMR DIADE, IRD, 911 avenue Agropolis, BP64501, 34394 Montpellier cedex 5, FRANCE

²Africa Rice, 01 BP2031, Cotonou, BENIN

Abstract

Rice yellow mottle virus (RYMV) is one of the main pathogen of rice in Africa and Madagascar. A single resistance gene, encoding a translation initiation factor is known in *Oryza sativa*, the Asian species of rice, worldwide cultivated. By contrast, high resistance appears to be more frequent and more diverse in the African cultivated rice, *Oryza glaberrima*: several resistance alleles at *RYMV1* locus and a second gene, named *RYMV2*, controlling a recessive resistance, have been described in that species. The characterization of *RYMV2* is critical for the development of resistant varieties and the sustainable control of the disease.

RYMV2 was mapped in a region of about 30kb, using the resistant accession Tog7291. Four genes and several repeated elements are annotated on the corresponding sequence of the reference accession Nipponbare. Sequencing of the region revealed that Tog7291 is characterized by few SNPs or short deletions compared to the susceptible *O. glaberrima* accession CG14. Most of those mutations occurred in non-coding regions and repeated elements, except a 1bp-deletion in the first exon of gene *Os01g68970*, that results in a frameshift and a stop codon, and probably a non-functional protein. Gene *Os01g68970* is homologous to *Arabidopsis thaliana* *CPR5* gene, involved in particular in defense mechanisms and senescence. A CAPS marker has been designed on this deletion and a collection of more than 350 rice accessions was screened. The mutation is present in five additional *O. glaberrima* resistant accessions, while it is absent in all the susceptible accessions tested. *Os01g68970* is therefore considered as the best candidate gene for *RYMV2*.

In parallel, a QTL of resistance was identified in the same chromosomic region using the IR64 x Azucena population. Fine mapping of this QTL using advanced back-crossed progenies pinpointed a candidate region of 210 kb including *RYMV2*, suggesting that the same gene may be involved both in partial and high resistance depending of the allele or genetic background. *Os01g68970* sequence comparison between the parental accessions IR64 and Azucena showed some amino-acid substitutions and deletions of 30 and 40 nucleotides in the promoter region that may be responsible of differences in the regulation of *Os01g68970* expression.

RYMV2 resistance-breakdown has been studied using Tog7291 accession and isolates representative of RYMV variability. Preliminary results suggested contrasted ability of the different isolates in *RYMV2* resistance-breaking ability.

Keywords: rice virus resistance gene QTL resistance-breakdown

Session 4
Socio-economic issues
related to the use of resistant
varieties
and their deployment in agro-
systems

Path-dependency and socio-technical lock-in: the difficult quest of relevant innovations

Baret Ph.

Earth & Life Institute, Université de Louvain, Belgium

Abstract

Actions against plant diseases are based on a multiplicity of solutions. A naive and optimistic discourse on this diversity of pathways states that all innovations are welcome to answer an agronomic issue. Such a posture is based on two assumptions: (1) all innovations have an equal probability of emergence and (2) innovations are always synergistic. A systemic analysis of two cases, scab in apple (Vanloqueren and Baret, 2004) and fusarium in wheat (Vanloqueren and Baret, 2008), contradicts these assumptions. Indeed, innovation development is constrained by different factors such as availability of funding, previous knowledge, financial and reputational interests (Merton, 1968), and visions of progress. In consequence, a path dependency phenomenon constrains the emergence of innovations: most of the new technical solutions are adaptation of extant technologies. Moreover, agricultural systems are multi-agents: farmers, advisers, public and private research, value chain. An analysis of the organisation of this network of actors and the norms they share resorts to the concept of socio-technical regime (Geels, 2004). Dominant socio-technical regimes are prone to lock-in: the system excludes some potential solutions as they doesn't fit the interests and the conceptions of the members of the network (Stassart and Jamar, 2008; Vanloqueren and Baret, 2009). Due to the limited nature of financial and knowledge resources, potential solutions are in competition. As a matter of fact, the implemented solution in the fields and orchards are not always the most relevant ones and, in some cases, the most relevant solutions are ignored or locked-out.

Quest for more relevant innovations implies an analysis starting from the problem and not from the existing solution, an understanding of the nature of the socio-technical network in place and subsequent transition strategies (Smith et al., 2005; Geels and Schot, 2007) and the combination of technical and non technical innovations.

Keywords: Innovation, socio-technical regime, path-dependency, lock-in.

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Pest Resistance Management: an economic perspective

Desquilbet M.

Toulouse School of Economics, GREMAQ, INRA

Marion.Desquilbet@toulouse.inra.fr

Abstract

This presentation gives a perspective on the main findings of the economic literature dealing with pest resistance management and on questions for future research. Resistance to pest damage is embedded in some crop varieties, which thereby have an economic advantage over their conventional non-resistant counterparts. But this advantage may be lost over time, as selection pressure causes pest populations to adapt to this resistance. The economic literature has mainly illustrated the examples of resistance to a chemical pesticide or to an insect-resistant transgenic variety (as well as the related case of weed adaptation to a herbicide-tolerant transgenic crop variety on which a total herbicide may be applied). It has explored how to best design the use of pest-toxic varieties over time, which involves an intertemporal trade-off between controlling the pest population now and preserving its susceptibility to the pest-toxic crop in the future. It has also analyzed how to regulate this use, with regulation being motivated by the common-pooled nature of pest population and pest resistance, two detrimental resources exploited by farmers under open access. Besides, it has tackled the issue of how the interest of a social planner in preserving the resistance of a pest-toxic variety compares with that of a monopolist selling the crop variety with patent rights. At last, the literature has looked into the question of possible increasing returns to adoption in disfavor or in favor on pest-toxic varieties. Vanloqueren and Baret (2008) illustrate path-dependent processes and lock-in factors playing against the diffusion of multi-resistant wheat cultivars. In the case of herbicide-tolerant transgenic varieties, which allow the use of a total herbicide to combat weeds, on the contrary, some weed scientists warn on some path-dependence factors that play in favor of increased herbicide use over time to control weeds with herbicide-tolerant varieties (Mortensen et al., 2012).

Keywords: Pest resistance, intertemporal optimization, common property resource, monopoly, lock-in

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Resistance management and information acquisition: an economic analysis

Lemarié S.¹; Marcoul Ph.²

¹INRA, GAEL, Grenoble, France

²Department of Resource Economics and Environmental Sociology, University of Alberta, Edmonton, United States of America

Abstract

The development of technologies aimed at containing pest damages in agriculture has led to major productivity gains during the last fifty years. Among these technologies, pesticides and resistant varieties are arguably effective ways of increasing crop yields. However, their indiscriminate utilization is not without controversy and raises important questions. For instance, it is now widely recognized that an intensive use of these products may not be optimal because pest may then become rapidly resistant to them. In other words, while intensive use is economically beneficial in the short-run because it decreases damages, it is largely unfavorable in the medium or long-run because pest resistance decreases their efficiency.

This problem has been addressed in the agricultural economic literature. Based on resource extraction modeling, it is shown that it is optimal to balance short-run gains and long-run costs by decreasing the intensity of use in the short term. It is also shown that when the pest is mobile, the sensitivity of the pest becomes a common pool resource. As a consequence, even if one farmer knows that he overuses the technologies, there are no economic incentives for him to moderate this use because he will capture only a very small fraction of the benefit that is generated. All these analyses are developed under the assumption that farmers and regulator have perfect information regarding the characteristic of the pest population at any time. This assumption is unrealistic because the exact level of sensitivity in the pest population remains unknown for the most. The aim of this paper is to analyze the economic impact of the information acquisition on pest population, to manage resistance in a more sustainable way.

We develop a simple economic model that illustrates the dynamics of learning and its impact on pest resistance management. Our model features two growing seasons during which a grower must decide the intensity of use of the pest control technic (e.g. a resistant variety, a pesticide). These two growing seasons correspond to the life cycle of these inputs over which the farmer must optimize their use. We assume that the grower is unsure of the efficiency of the pest control input because it depends on the (unknown) pest population sensibility to pesticide. In this framework, his control decision is the result of several competing forces. He needs to control damages at the current period but must also learn about pest resistance to enhance future control decisions knowing that the pest is more likely to develop resistance when these inputs are used more intensively. Two scenarios are considered depending on whether the input is sold by a monopolistic firm or is available at a lower competitive price.

We show that acquiring information allow the grower to better adapt its use at the first period, depending on the level of resistance in the pest population. The average resistance of pest at the second period is therefore decreased and the economic surplus over the two periods increases. In other words, acquiring information is economically beneficial because it enables the grower to make a more accurate choice. When the input is sold by a monopoly during the two periods, the monopoly has an interest to increase the price at the first period to maintain the efficacy of the input at the second period. However, this property does not eliminate the benefit of acquiring information. Indeed, acquiring information is also economically beneficial in a monopoly context. Moreover, it alters the allocation of the economic surplus between the growers and the firm.

Keywords: Agricultural, Pest resistance management, Economics, Information value, Bayesian Learning, Market power

Cognitive alignment, Accommodation and Percolation: Three ways of extension of resistant varieties and sustainable farming Methods

Hochereau F.

INRA SenS, France

Abstract

Historically speaking; agricultural modernisation has been based on artificial crop systems centered on the standardization of farming practices. Today, the demand for safe agricultural products combined with an environmentally friendly framework leads to rethinking how to innovate in agriculture. In this study we examined the process of legitimating and extending sustainable agriculture through the elaboration and experimentation of **resistant wheat varieties and their deployment in agro-systems**.

We have done this in two ways: first in the light of the history of the interactions between scientists and different kinds of expertise to test the performance of resistant varieties associated with low chemical input farming methods; secondly; in studying how this knowledge spreads in different ways according to specific issues in three “agro-socio-ecological” contexts. The first two contexts are located in two northern France counties with high potential crop yields, which differ in the predominance of economical or political constraints. The latter situated in a southern France county is more focused on environmental issues, because of the development of tourism there.

In this study, we question the trend described by Collins and Evans (2003) from top-down to bottom-up driven production of knowledge in explaining why the problem of extension emerged and how the different actors involved tried to deal with it.

The results of our analysis show three learning mode in innovative farming methods diffusion, according to local priorities, expertise of extension agents and the risk culture of the farmers involved. In “Cognitive alignment”, farmers and extension agents ensure the assessment of scientific knowledge. In the “Accommodation way”, farmers and extension agents shift the emphasis of sustainable agriculture from the farming methods issues to the certification of what a sustainable farm should be. In the “Percolation way”, scientific knowledge is less the frame of the sustainable agriculture development than single cognitive resources that allow farmers to draw on context-specific experiential understandings of their own agro-ecosystems and allow them to complete their practices within agro-ecological schemes.

The author concludes that these differing visions of sustainable farming methods and appropriateness of resistant varieties, as well as controversial views of what is sustainable agriculture and how agriculture has to be changed, form coalitions which may play a significant role in shaping the future of agriculture.

Keywords: ways of knowledge extension, legitimation of innovation, bottom-up production of knowledge, sustainable farming Methods, uses of resistant varieties

Local management of phoma stem canker of winter oilseed rape and resistance sustainability

Hossard L.^{1,2,3,4}; Souchere V.^{3,4}; Pelzer E.^{1,2}; Pinochet X.⁵; Jeuffroy M.H. ^{1,2}

¹INRA, UMR211 Agronomie, F-78850 Thiverval-Grignon, France

²AgroParisTech, UMR211 Agronomie, F-78850 Thiverval-Grignon, France

³INRA, UMR1048 SADAPT, F-78850 Thiverval-Grignon, France

⁴AgroParisTech, UMR1048 SADAPT, F-78850 Thiverval-Grignon, France

⁵Cetiom, BP-04, F-78850 Thiverval-Grignon, France

Abstract

To sustainably manage and control disease epidemics, three technical levers can usually be used: genetic, agronomic and chemical. For phoma stem canker of winter oilseed rape (WOSR), caused by *Leptosphaeria maculans*, the intensive use of the genetic control method can jeopardize the sustainable control of the disease: indeed specific (qualitative) resistances may be quickly overcome (Rouxel et al., 2003), depending on both the characteristics of pathogen population and the genetic background of the cultivars harbouring the new specific resistance (i.e. presence and type of quantitative resistance). Managing the pathogen population (size and structure towards avirulence gene) is thus crucial to control efficiently and sustainably phoma stem canker, and should combine agronomic and genetic levers, in space and time.

The activation and organization of the different control methods are conditioned by local cropping conditions (i.e. pedoclimatic conditions and territorial organization, including stakeholder local organization). Therefore two contrasted regions (Centre and Picardie) were chosen to design with stakeholders the possible evolutions for cropping systems including winter oilseed rape, in face of credible context changes they imagined. Activated leverages differed between regions and future contexts, e.g. rotation length (to determine WOSR frequency) sowing date and density, cultivar, organic nitrogen fertilization, fungicide use, post-harvest residues management and weather context (current vs. a priori favourable to phoma stem canker, i.e. with wet and mild autumns). The scenarios designed in both regions were then tested, on a local simulation support map (about 17 km² for each map), with the spatially-explicit model SIPPOM-WOSR (Lô-Pelzer et al., 2010) to appraise their efficacy for phoma stem canker management and resistance sustainability. These scenarios (3 replicates to account for the impact of cultivar/practices location) were assessed using three indicators calculated for the last of the five years of simulation at the small region scale: the size of the pathogen population, the WOSR yield losses due to the disease and the frequency of virulent pathotypes toward the tested specific resistant gene (here Rlm7-gene, cultivated in both regions and not yet overcome).

For these three criteria, linear models were used to assess the effects of cropping practices and weather conditions for each regional future context imagined by stakeholders. Population size was mostly explained by changes in WOSR acreages, cultivar landscape composition and tillage. Yield losses variations were mostly explained by weather conditions and cultivars. Frequencies of virulent pathotypes on Rlm7-cultivars were mainly explained by cultivar composition of the landscape and WOSR acreages, but with globally low explanatory values (< 50%) as compared to the two other assessment criteria (>80%). Comparison of the two regions highlighted the differences in potential leverages (cropping practice) that may be activated in front of a context change and consequences for management of phoma stem canker and resistance sustainability. Results for the variable concerning the sustainability of Rlm7-gene highlighted the necessity to consider detailed location factors, e.g. neighbourhood effects, suggesting territorial organization as an important factor for resistance sustainability and thus a necessary local cooperation for an efficient disease control.

Keywords: spatial simulation model, phoma stem canker, winter oilseed rape, cropping practices, resistance sustainability

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Controlling cyclic epidemics on the crops of the agro-ecosystems by resistant varieties: when should one simplify the reality?

Bousset L.

INRA, UMR1349 IGEPP, F-35653 Le Rheu, France

Abstract

The control of epidemics is focal point of several scientific disciplines because the way crop yield is reduced by epidemics depends on plants, pathogens, environment, and humans. This induces the need to formalize epidemics in order to control them. In agro-ecosystems, human actions exacerbate homogeneities alternating with sharp discontinuities on scales of time and space. The dynamics of cyclic epidemics takes discontinuities into account. This allows decomposing control at the field and at the landscape levels into goals to reach, corresponding to the components of the pluriannual dynamics of epidemics. The use of genetic resistance, qualitative or quantitative, indeed is one of the crop protection tactics. Because it acts on epidemiology, breeding can contribute to the aim of increasing the durability potential of resistances. We will describe how the adaptation of pathogen populations depends not only on the epidemics on one field during one season, but instead on a pluriannual dynamics. Formalization of epidemiology including transmission of inoculum between fields and between years allows exploiting more widely the capacities of breeding (Bousset et al. 2011). The prospect of our conceptual work (Bousset and Chèvre 2012) is allowing for a better definition of roles towards easier interdisciplinary exchanges. The networking between concepts by relating them to the dynamics of pluriannual epidemics in agro-ecosystems yield the benefits to combine tactics issued from different scientific disciplines for each of the attributed aims. The high level of complexity following from the large number of interacting elements leads to an antagonism: theory has to be inclusive of all aspects but studies have to be parsimonious. Achieving the articulation of the whole set of disciplinary concepts allows to realize that simultaneous understanding of all factors is beyond reach, therefore beyond prediction. There is no single, general and unique solution that can be studied and reached. On the contrary, the optimal balance depends on the local situation in time and in space. By local, we refer to the cropped species, the biology of pathogens, the production situations and the available tactics. The question that arises is: should one simplify upstream and look for one unique strategy to be recommended in a centralised manner, or simplify downstream and allow for the decentralised implementation of local strategies? We will present how cyclic epidemics are different from polyetic epidemics and could therefore be managed in a different way, even if it is not excluded that decentralised strategies could also be appropriate for polyetic epidemics.

Keywords: Epidemiology, plant resistance, efficiency, crop protection tactics, modelling

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Abstracts of Poster Sessions

Session 1
Impact of plant disease resistance
on the structure and evolution
of pathogen populations

Differential selection pressures exerted by resistance QTLs on a pathogen population: a case study in apple - *Venturia inaequalis* pathosystem.

Lê Van A.¹; Caffier V.¹; Lasserre-Zuber P.¹; Chauveau A.²; Brunel D.²; Le Cam B.¹; Durel C.E.¹

¹INRA, UMR1345, Institut de Recherche en Horticulture et Semences (INRA/AgroCampus-Ouest/Université d'Angers), SFR 4207 QUASAV, BP 60057, F-49071 Beaucouzé, France.

²INRA, US 1279, Etude du Polymorphisme des Génomes Végétaux, BP 5721, F-91057 Évry, France

Abstract

Understanding how pathogens evolve according to pressures exerted by their plant hosts is essential to derive strategies aiming at a durable management of resistant cultivars. The spectrum of action of the resistant factors in the partially resistant cultivars is thought to be an important determinant of resistance durability but whether the pressures exerted by quantitative resistance are different according to the spectrum of action has not been demonstrated so far. Here we investigated selection pressures exerted by apple genotypes harbouring various quantitative resistance loci (QRLs) against the scab disease agent *Venturia inaequalis*. Using quantitative pyrosequencing technique we monitored isolate proportions in a mixed inoculum on sporulating leaves of a mapping population segregating for QRLs. This population had been previously inoculated independently with each isolate of the mixed inoculum to qualify the spectrum of action of detected QRLs. We observed that broad spectrum resistance factors did not exert differential selection pressures on the mixed inoculum while narrow spectrum resistance factors favoured some isolates in the mixture compared to the susceptible host genotypes. Our result suggests that the management of resistance cultivars should be different according to the spectrum of action of their resistance factors. Pyramiding of broad spectrum factors or using a mixture of apple genotypes carrying narrow spectrum resistance factors exerting differential selection pressures are two possible strategies aiming at minimising resistance erosion.

Keywords: selection pressure, *Venturia inaequalis*, Malus x domestica, quantitative resistance, broad spectrum resistance, quantitative pyrosequencing

Modeling crop disease risk and variety-pathotype interactions at the regional scale by a Bayesian approach

Blanchard D.¹; Monod H.¹; Lannou C.²; Goyeau H.²

¹UR 341 MIAJ, F-78352 Jouy-en-Josas

²UMR 1290 BIOGER, F-78850 Thiverval Grignon

Abstract

The use of genetic diversity for controlling epidemics and reducing our dependency to pesticides is a widely shared idea in plant pathology, but whether this is applicable at the scale of cultivated landscapes is still an open question and requires developing a better knowledge of the evolution over years of pathogen populations in varietal landscapes.

In a first study of the spatiotemporal dynamics of the French wheat rust populations on different wheat varieties, we shown that the varietal composition of the landscape influences the composition of the pathogen population and, in returns, the observed resistance level of the varieties that are the most widely used by the farmers (Papaïx et al., 2011). In a further exploration of the datasets, we assumed the existence of an autoregressive character in the pathotype distribution (i.e. that the pathotype distribution of year n was influenced by its distribution of year n-1) and we refined the scale of study by decomposing the area under investigation into smaller regions.

We jointly analyzed two large data sets. The first one describes the wheat leaf rust (*Puccinia triticina*) pathosystem at the scale of the French territory, divided into seven regions. The second one contains the frequencies of the main varieties of wheat each year and in each region. A selection of the data was first performed. We selected the varieties that are the most represented in the French agricultural landscape and the 6 major pathotypes of the leaf rust population. This selection was based on homogeneity criteria applied over the whole period (1999 – 2010) and across all regions. The model was analyzed with a Bayesian approach. Uniform *a priori* were attributed to the model parameters.

We showed that the frequencies of pathotypes on a variety in year *n* were influenced by both the frequency of that pathotype in year *n-1* and by the varietal structure of the landscape in year *n*. We confirmed the existence of quantitative differences in aggressiveness between varieties and pathotypes that contribute to the pathogen population structuration.

The regionalization of the model proposed by Papaïx et al. (2011) allowed refining its explanatory quality and confirmed the interest of analyzing these populations at smaller scales. Our next objective is to test the predictive capacity of the model for estimating the resistance level of largely grown varieties. This may open new possibilities for developing variety management strategies at the scale of cultivated landscapes.

Keywords: Bayesian modeling, crop resistance durability, hierarchical model, landscape epidemiology, regionalization, population genetics, *Puccinia triticina*, wheat leaf rust.

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New insights on virulence and evolutionary dynamics of the *Ralstonia solanacearum* species complex –keys and challenges in the search of durable resistance in *solanaceae*

Wicker E.¹; Lebeau A.¹; Pensec F.¹; Daunay M-C.²; Dintinger J.²

¹CIRAD, UMR « Peuplements Végétaux et Bioagresseurs en Milieu Tropical »(PVBMT), 7 chemin de l'IRAT, 97410 SAINT PIERRE Cédex (La Réunion)

²INRA, UR « Génétique et Amélioration des fruits et Légumes », Domaine St Maurice, BP 94, 84143 Montfavet cedex

Abstract

Bacterial wilt, caused by the soilborne betaproteobacterium *Ralstonia solanacearum*, is a major disease of solanaceous crops, throughout tropical and subtropical areas, and emerging in temperate regions. If genetic resistance is still considered as the most promising control measure, breeding for resistance to *Ralstonia solanacearum* (*Rsol*), has been hindered for decades by the scarcity of high level resistance sources, strong genotype x environment interactions, and the huge genomic and phenotypic plasticity of the pathogen. *Ralstonia solanacearum* is a species complex composed of four phylotypes related to geographical origins (I: Africa-Asia, II: American, III: Africa, IV: Indonesia). Solanaceae-pathogenic strains belong to all four phylotypes, but most epidemiologically active strains belong to phylotype I, IIA, IIB, and III, phylotype I being the most widespread lineage.

Extensive screenings of resistant accessions of tomato, eggplant and pepper towards a worldwide *R.sol* core-collection, lead to formalize grain Solanaceae-*Rsol* interactions into six main virulence profiles, and allowed to identify highly resistant accessions, mainly in eggplant and pepper. Among them, the eggplant MM960 was demonstrated to carry a phylotype I-resistance major dominant gene (*Ers1*) and

two partial resistance QTLs with strain-specific expression. We identified patterns of strain –specificity, but found no evidence of phylotype-specificity; phylotype I containing four of the six pathoprofiles.

Searching for durable resistance implies both the identification of bacterial genes responsible for avirulence and virulence, and assessment of the pathogen evolutionary potential. Considering “immune” accessions (five eggplants, two peppers, one tomato) giving true incompatible interactions (no wilt and no colonization), we then searched for genetic factors associated with bacterial virulence and avirulence using comparative genomics hybridization (CGH) data. We thus identified 61 type III effectors fairly to highly associated to avirulence to eggplants Dingras, SM6, Ceylan, Surya, and MM960, peppers CA8 and Perennial, and tomato HW7996. Interestingly, some of these effectors were elsewhere shown to contribute to bacterial fitness. Focusing on the accessions MM960 and Dingras, less than 10 genes were highly associated with avirulence, among which *popP2*, avirulence gene interacting with the RRS1-R resistance gene in *Arabidopsis thaliana*.

We inferred evolutionary dynamics of the *R.solanacearum* species complex (RSSC) at a global scale, performing multi-locus sequence analysis (MLSA) and gene genealogies on a worldwide collection. Although recombination was found ubiquitous within the RSSC, we observed phylotype-specific recombination rates and demographic histories. Phylotype I (and, to a lesser extent, phylotype III) is highly recombinogenic, and carries molecular signatures of a recent and rapid demographic expansion. Phylotype II is actually composed of two evolutionary lineages, one (IIA) being moderately recombinogenic and expanding, and the other (IIB) being clonal and poorly expanding. Taken together, these findings strongly suggest that phylotype I is the most prone to adapt rapidly to new hosts and varieties, thank to its recombination and mutation rates, capacity to disseminate, and large virulence range.

To decipher evolutionary dynamics leading to adaptation at the local, field scale, an experimental evolution trial is in course to monitor the impact of *Ers1*-carrying eggplant MM960 successive crops on the population structure and putative avirulence genes evolution.

Keywords: population genetics, virulence, *Solanum*

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Durability of the partial resistance to blast controlled by several major QTL in upland rice and efficacy of the introgression of resistance genes from upland to paddy rice cultivar

Zenbayashi-Sawata K.¹; Kito H.¹; Ashizawa T.²; Koizumi S.²

¹NARO Tohoku Agricultural Research Center (NARO/TARC)

²NARO Agricultural Research Center (NARO/ARC)

Abstract

Upland rice cultivars are valuable genetic resources of partial resistance to blast for breeding of paddy rice in Japan. These resistance genes have been detected by QTL analysis. Consequently, several genes have already been identified and cloned. The near-isogenic lines of paddy rice introduced these genes by marker-assisted selection have been developed, while the strategy for their sustainable utilization has not yet established.

Pi34, one of the genes described above, conferring quantitative resistance, was precisely mapped on the chromosome11, and no R-gene (which has NB and/or LRR domains) was found in the mapping region(1).

The segregation analysis of F1 progenies crossing rice blast strains *Y93-245c-2* and *IBOS-8-1-1*, which show weak and strong aggressiveness against *Pi34* respectively, revealed that *Y93-245c-2* has a single AVR gene correspond to *Pi34*. That is, a gene-for-gene relationship between a resistance gene associated with severity of disease (quantitative trait) and pathogen aggressiveness was confirmed (2).

Because a rice blast strain *IBOS8-1-1* was isolated from an upland rice cultivar, we suppose that blast strains isolated from upland rice may possess strong virulence (aggressiveness) against other partial resistance genes on upland rice. As a result of the investigation for virulence of rice blast isolates from upland rice on a breeding nursery at Ibaraki Agriculture Institute, Japan, a few isolate showed strong aggressiveness to *Pi34*. Interestingly, one of these isolates severely infected cv. 'Sensyo' which have four QTLs of resistance including *Pi34*. From these results, a question arises whether partial resistance genes derived from upland rice may not durable.

To find a clue of the answer, the population structures of rice blast fungus on upland and paddy rice were compared. For upland rice, the farmers usually get the seeds for next cultivation by on-farm seed production. They seldom control diseases with chemicals in the fields. On the other hand, the proportion of seed use from on-farm seed production to total seed use in paddy rice is generally very low. Almost all farmers perform seed sterilization, as well as chemical control in the fields. Therefore, bottleneck effect during overwintering on blast population on upland rice may be smaller than that on paddy rice. To understand the evolutionary forces to change blast population on quantitative resistant rice cultivars, we started to measure the parameters of fitness and genetic drift of blast populations.

Keywords: blast, partial resistance, upland rice, gene-for-gene

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Impact of host domestication on the genetic structure of the poplar rust fungus

Frey P.¹; Xhaard C.¹; Barrès B.^{1,2}; Fabre B.¹; Halkett F.¹

¹INRA, Université de Lorraine, UMR1136 Interactions Arbres - Microorganismes, F-54280 Champenoux, France.

²INRA, Université de Bordeaux, UMR 1202 BIOGECO, F-33662 Cestas, France

Abstract

The quantification of gene flow between populations of pathogens infecting cultivated and wild host plants is of great importance for the management of disease resistance (Stukenbrock & McDonald, 2008). The pathogen populations from the wild pool can be a reservoir of genetic diversity from which can emerge new virulent individuals. In contrast, the introgression in the wild pool of virulence factors previously selected in the cultivated pool may constitute a threat for conservation of biodiversity of the wild plant. Poplar rust, caused by *Melampsora larici-populina*, has become the main constraint for commercial poplar cultivation. The poplar cultivar 'Beaupré', carrying a rust qualitative resistance gene, has been widely planted in France since 1982 and remained immune for 12 years. Following its resistance breakdown in 1994, severe rust epidemics and high economic losses occurred. In this study we studied the effect of the massive deployment of a resistant cultivar in the French poplar cultivation landscape on the population structure of the pathogen in the wild pool. In total, 1,050 individuals sampled from the wild host, *Populus nigra*, in 23 sites in France were phenotyped to determine their virulence profile, and 476 individuals were genotyped using 25 microsatellite markers. Bayesian assignment tests revealed three genetic groups. Group 1, widely distributed in the Northern half of France, gathered most virulent individuals and displayed evidence for selection and drastic demographic changes resulting from breakdown of the poplar cultivar 'Beaupré' (Xhaard *et al.*, 2011). Group 2 comprised individuals corresponding to ancestral populations of *M. larici-populina* naturally occurring in the native range, especially in some geographically remote regions, such as the Alps (Xhaard *et al.*, 2012). Group 3 displayed the hallmarks of a long lasting asexual reproduction, which has never previously been demonstrated in this species. This work illustrates how the massive deployment of a qualitative resistance gene in the cultivated pool can change the genetic structure of populations of a pathogen in both the cultivated and the wild pool of the host plant.

Keywords: resistance deployment, selection, disease spread, gene flow, rust fungus

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Contribution of microbial core-collections and exploitation of polygenic resistances to the development of *phytophthora* control strategies in solanaceous crops

Kuhn M-H.^{1*}; Massire A.^{2*}; Cantet M.²; Damidaux R.²; Gibowski E.¹; Ruellan Y.²; Lefebvre V.^{2}; Panabières F.^{1**}**

Marie-Line Kuhn^{1*}, Anne Massire^{2*}, Mélissa Cantet², René Damidaux², Elvina Gibowski¹, Youna Ruellan², Véronique Lefebvre²⁼, Franck Panabières¹⁼

¹UMR INRA/CNRS/UNS 1355 Institut Sophia Agrobiotech, 400 route des Chappes, 06903 Sophia-Antipolis, France;

²INRA, UR 1052 Génétique et Amélioration des Fruits et Légumes, Domaine St Maurice, Allée des Chênes, BP 94, 84143 Montfavet cedex, France.

*contributed equally to this work;

**contributed equally to this work

Abstract

Oomycetes form a diverse group of fungus-like eukaryotic microorganisms that include saprophytes as well as pathogens of animals and some of the most devastating pathogens of dicotyledonous plants. Among them, few species of the genus *Phytophthora*, such as *P. capsici* and *P. infestans* cause enormous economic damage on Solanaceous crops. Major genes have been identified in tomato to confer resistance against *P. infestans*, but were rapidly overcome by virulent populations. Pepper resistance towards *P. capsici* is provided by several quantitative trait loci (QTLs). Efficient exploitation of such polygenic resistance sources in plant breeding programs requires a good evaluation of their spectrum and durability. This requires a precise knowledge of the diversity of pathogen populations (with a focus on virulence, aggressiveness and host range) and some of their adaptive traits, such as changes in virulence or adaptation to prevalent cultivars. Theoretically, the use of *Phytophthora* core-collections reflecting the overall intraspecific diversity would help to assess the *a priori* durability of resistance sources.

We initiated a study to evaluate the potential sustainability of polygenic pepper and tomato resistances to *Phytophthora*. It implied i) to estimate the diversity of *P. infestans* and *P. capsici* collections and their evolution over time; ii) to develop diverse quantitative and qualitative pathogenic assays to identify the various components underlying polygenic resistances. Collections included isolates from various geographic origins. Genetic diversity was investigated using a combination of neutral markers and genes under positive selection. First results on *P. capsici* reveal significant variations in the structure and dynamics of regional populations, and suggest that *P. capsici* core-collections may be of local interest. We also showed that *P. infestans* populations are diverse but structured. Hence, strains isolated on tomato or potato differ at the genotypic and phenotypic levels. The use of *P. infestans* core-collections in breeding programs therefore requires gaining knowledge on the structure and epidemiological characteristics of natural pathogen populations collected on tomato.

Keywords: Resistance, *Phytophthora* spp., pepper, tomato, core-collection, plant breeding.

Erosion of quantitative resistance loci related to an increase in pathogen aggressiveness and consequences on the efficiency of their combination with a major gene

Caffier V.¹; Lasserre P.¹; Giraud M.²; Lascostes M.³; Le Cam B.¹; Durel C.E.¹

¹INRA, UMR1345, Institut de Recherche en Horticulture et Semences (INRA, Agrocampus-Ouest, Université d'Angers), SFR 4207 QUASAV, F-49071 Beaucouzé, France

²Centre CTIFL de Lanxade, 28 Route des Nebouts, F-24130 Prignonrieux, France ³ CRRG, Centre Régional de Ressources Génétiques, Ferme du Héron, Chemin de la ferme de Lenglet, F-59650 Villeneuve d'Ascq, France

Abstract

Quantitative resistance loci (QRL) are generally considered as more durable than major resistance genes. However, few experimental data are available on the stability versus erosion of the QRL efficiency in the field, and on the impact of the QRL on the evolution in pathogenicity of pathogen populations. In the apple scab pathosystem, many QRL have been identified and mapped. In the present study we focus on the combination of two QRL: F11 and F17. Our aims were (1) to evaluate the durability of QRL and their impact on the aggressiveness of *Venturia inaequalis* populations, and (2) to evaluate if the combination of these 2 QRL with the major gene *Rvi6* (= *Vf*) improves resistance durability. A progeny segregating for these QRL was planted in orchard in 3 sites in France. Scab disease was observed on each apple tree over a 6-year period. *V. inaequalis* isolates were sampled at different dates, and their aggressiveness (number of lesions produced per cm² of leaf, percentage of scabbed leaf area) was assessed after inoculation in controlled conditions on apple genotypes carrying the QRL. Our results showed that in 2006 the efficiency of the QRL combination for controlling scab differed depending on the sites, and can be explained by a contrasting level of aggressiveness of the isolates in the different sites. Furthermore, in one site we observed an erosion of the efficiency of the QRL combination from 2006 to 2011. In this context of different regional evolutions in the efficiency of the QRL combination, we evaluated if the combination of these 2 QRL with the major gene *Rvi6* improves the durability of the resistance in these 3 sites. For *Rvi6*, 3 different situations were taken into account: no breakdown of *Rvi6*, complete breakdown of *Rvi6* from the beginning of the experiment, on-going breakdown of *Rvi6* within the course of the experiment. Our result showed that even in the "worst" situation, i.e. erosion of QRL and breakdown of major resistance gene, there was a stable and significant efficiency of the combination of QRL and major gene compared to QRL alone or major gene alone.

Keywords: scab, apple, quantitative resistance, aggressiveness, erosion, pyramiding

Pathogenicity of genetically different *Sclerotinia sclerotiorum* isolates on important crops

Reza Ahmadi M.¹; Javan-Nikkhah M.¹; Ali Aghajani M.A.²; Reza Naghavi M.³

¹Department of Plant Protection, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

²Department of Plant Protection, Agriculture & Natural Sciences Research Institute of Golestan Province, Gorgan, Iran

³Department of Agronomy and Plant Breeding, College of Agriculture and Natural Resources, University of Tehran, Karaj, Iran

Abstract

Sclerotinia sclerotiorum is one of the most important and devastating plant pathogens with world-wide distribution. The fungus infects more than 500 species of plants without being known as host specificity in pathogenicity. Many researchers formerly believed there was no resistance to this pathogen. In this study, the genetic diversity of sixty *S. sclerotiorum* isolates obtained from different host species (populations) including rape-seed, soybean, sunflower, lettuce, cabbage, tobacco and sagebrush in Iran was examined using five polymorphic microsatellite loci. Out of 60, 30 isolates were selected based on their hosts and geographical distribution for assessing the pathogenicity on the most cultivated variety of important crops involving rape-seed cv. Hyola, cabbage cv. White, soybean cv. Dpx, lettuce cv. Varesh and sunflower cv. 8-light. Among above-mentioned host populations, percentage of polymorphic DNA bands ranged from 36.84% to 89.47% demonstrated high level of genetic diversity. Analysis of molecular variance revealed that 81% of the variability among isolates was found within subpopulations. The cluster analysis based on UPGMA algorithm and Jaccard's coefficient showed that most isolates belonging to a plant family were grouped in the same cluster or closed clusters. The Nie_'s genetic identity illustrated that populations of lettuce and sunflower and/or cabbage and rape-seed were genetically close together. Ewens-Watterson tests did not determine significant deviations of haplotype ($P \leq 0.05$) in all studied populations, indicating that the observed variation in microsatellite loci was not affected by natural selection. According to pathogenicity results, the analysis of variance ($P \leq 0.05$) showed significant difference among isolates. Mean comparison of leaf spot sizes resulted in 30 isolates and seven host populations in 20 and five homogenous statistical groups, respectively. In spite of high variation, pathogenicity and genetic diversity of isolates were not related together. Component variance analysis estimated that the effect of isolate pathogenicity, plant susceptibility and isolate \times plant interaction were 31%, 21% and 48%, respectively. In addition, susceptibility of examined greenhouse plants revealed two homogenous groups, including lettuce and sunflower (Asteracea) as sensitive and cabbage and rapeseed (Brassicaceae) and soybean as resistant groups to *S. sclerotiorum* isolates. Taken together, assessing of plant susceptibility to *S. sclerotiorum* among various plant families would produce useful information on identifying resistance source.

Keywords: *Sclerotinia sclerotiorum*, Genetic diversity, Pathogenicity, Plant susceptibility, Pathogen \times Plant interaction

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Genetic structure and chemotype composition of *F. graminearum* in forage maize in the United Kingdom

Basler R.^{1,2}; Edwards S.²; Thomas. J.¹

¹National Institute of Agricultural Botany, Huntingdon Road, Cambridge, CB3 0LE, United Kingdom

²Harper Adams University College, Newport, TF10 8NB, United Kingdom

Abstract

Fusarium graminearum is an important maize pathogen that is capable of producing three important mycotoxins; deoxynivalenol, nivalenol and zearalenone as well as other toxigenetic compounds. Six hundred and sixty-six plants were sampled from 10 forage maize fields in 2011 in the United Kingdom for the incidence of *Fusarium* contamination in the stalk and kernels. The predominant isolates in the maize samples were *F. culmorum* and *F. graminearum*. Morphological characteristics and a species-specific assays were used to confirm the isolates in the *F. graminearum* complex. Simple sequence repeats were used to determine genetic diversity and PCR assays for functional Tri13 and Tri7 genes were used to classify genotypes of 15-acetyldeoxynivalenol, 3-acetyldeoxynivalenol and nivalenol chemotypes. The diversity of metabolite production from chemical analysis will be used to determine if a relationship exists with the genotype diversity of the *F. graminearum* isolates from the field.

Keywords: *Fusarium*, mycotoxin, maize

Domestication of *rhizobacteria* as biocontrol agents

Kydraliev K.A.¹; Mamytova B.A.²; Muratov V.S.³; Khudaibergenova B.M.²; Jorobekova Sh.¹

¹Institute of Chemistry and Chemical Technology, NAS, Chui 267, Bishkek 720071, Kyrgyzstan, kamila.kydraliev@gmail.com

²Institute of Biology and Pedology, NAS, Chui 265, Bishkek 720071, Kyrgyzstan

³Institute of Applied Biochemistry and Machine-Building, K.Tsetkin 4, Moscow 125299, Russia

Abstract

In Kyrgyzstan there are several economically important plant diseases of commercial crops such as seedling blight, root rot, blackleg and stem rot, which cause serious damage to wheat, barley, maize, potato, melon, sugar beet and oilseed crops, especially in the irrigated areas. Although chemical fungicides and pesticides are used to control these diseases, development of natural humic substances and antagonistic microorganisms for biological control may be more successful. Thus, the performed

research and development program have been involved identifying natural humic substances and bacteria that produce novel metabolites that suppress or kill crop pests, determining the nature of the novel metabolites and using them towards the development of biocides. These substances and metabolites were formulated as commercial biocontrol products by Kyrgyz biotechnology companies as a viable first line of control of these crop diseases and possibly registered for release.

Screening, collection, identification and expression of stable and technological microbial strains living in soils and in the rhizosphere of weed - couch-grass *Elytrigia repens* L.Nevski and some commercial crops of Kyrgyzstan (tomato, corn) were conducted. A total of 132 bacteria isolated from the rhizosphere were assessed for biocontrol activity in vitro against phytopathogenic fungi including *Fusarium culmorum*, *Fusarium heterosporum*, *Fusarium oxysporum*, *Drechslera teres*, *Bipolaris sorokiniana*, *Piricularia oryzae*, *Botrytis cinerea*, *Colletotrichum atramentarium* and *Cladosporium* sp., *Stagonospora nodorum*. Biocontrol activity were performed using five methods: inoculation by radial and parallel streaks, by block and lawn, and "host – pathogen" on the cuts of wheat leaves. A culture collection comprising 64 potential biocontrol agents (BCA) against wheat and barley root diseases has been established. Rhizobacteria isolated have been studied at both bioassay, laboratory vegetation experiments and microfield trials scales as well. All the experiments confirmed not only high antagonistic potential of those *rhizobacteria* in relation to pathogenic fungi but also their plant growth-promoting activity. As a result, four bacterial strains selected from rhizosphere of weed - couch-grass *Elytrigia repens* L.Nevski were chosen as a core of collection pure cultures with high fungicidal and plant growth-stimulating potential. Partial determination of nucleotide sequence of 16S ribosomes of tested bacteria indicated that *Pseudomonas* and *Bacillus* species were the most dominant bacteria exhibiting biocontrol activity.

The laboratory technology of formulation of liquid and dry microbiological preparations on the basis of beneficial *rhizobacteria* was developed. Two drying formulation techniques have been tested: spray drying and low-temperature contact-convective drying in fluidized bed. The latter had higher beneficial effect in terms of high yield as compared to spray drying. Pilot batch of plant growth-promoting *rhizobacteria* was obtained. In study on isolation and purification of antibiotics resistant *rhizobacteria* mutants, ten spontaneous rifampicin- and streptomycin-resistant mutants of *Bacillus subtilis* BSRB-43 and rifampicin-resistant clones of *Pseudomonas* PsRB-7 were isolated. Rifampicin-resistant *Bacillus subtilis* RB-43 had a high antagonistic activity towards phytopathogen *Bipolaris sorokiniana*.

Keywords: Biocontrol; Humic substances; biocides

Analysing virulent and avirulent *Leptosphaeria maculans* isolates to understand R gene-mediated resistance in *Brassica napus*

Mitrousia G.K.; Huang Y-J.; Hall A. M.; Fitt B. D. L.

School of Life Sciences, University of Hertfordshire, Hatfield, Herts, AL10 9AB, UK.

Abstract

Phoma stem canker (*Leptosphaeria maculans*) is a major disease on oilseed rape (*Brassica napus*) in Europe (e.g. it causes losses to UK farmers of > £100M p.a.). Single major gene resistance (*R*) in *B. napus* against *L. maculans* is associated with a gene-for-gene interaction and a number of *R* genes are deployed in European commercial cultivars to control the disease (Delourme et al., 2006). In commercial practice, *R* gene-mediated resistance is often not durable since *L. maculans* populations change from avirulent to virulent. A single *L. maculans* gene *AvrLm4-7* interacts with two *B. napus* *R* genes (Parlange et al., 2009). The changes at the *AvrLm4-7* locus leading to virulence differ between *Rlm4* and *Rlm7* (Rouxel & Balesdent, pers. comm.). It has been suggested that *Rlm7* is more durable than other *R* genes commercially available (Clarke, pers. comm.). The fitness cost of virulence against *Rlm1* and *Rlm4* has been used to investigate durability of these *R* genes (Huang et al., 2010). This work aims to similarly

investigate durability of *Rlm7* in the UK.

Single pycnidial isolates were obtained from phoma spots on leaves of oilseed rape cultivars carrying the *Rlm7* gene (Roxet and Excel) in autumn/winter 2011/2012. Twelve of these UK isolates from Roxet and eight from Excel were characterised at the *AvrLm4-7* locus using cotyledon inoculation tests on cultivars carrying *Rlm4* or *Rlm7* genes. The phenotype was characterised as resistant (0–3.5 on the scale), intermediate (3.6–4.9) or susceptible (5.0–9). Unexpectedly, all isolates obtained from Excel and most isolates from Roxet produced resistant phenotypes on cultivars carrying the *Rlm7* gene. Only three isolates from Roxet produced an intermediate phenotype on cultivars carrying *Rlm7*. On cultivars carrying *Rlm4*, all isolates from Roxet produced a susceptible phenotype whereas the phenotype produced by isolates from Excel varied.

To investigate changes in fitness and aggressiveness associated with changes from avirulent to virulent at the *AvrLm4-7* locus, four isolates from Excel and four from Roxet that had produced resistant or intermediate phenotypes were characterised by inoculation onto cotyledons of a susceptible cultivar (Drakkar) and several cultivars carrying the *Rlm7* gene (Excel, Roxet and Hearty). Size of lesions and sporulation on the lesions were examined 17 dpi. All isolates produced susceptible phenotypes on Drakkar and resistant phenotype on the three cultivars with *Rlm7*. No statistically significant differences were observed between different cultivars carrying the *Rlm7* gene. Future work will include molecular characterisation of these isolates and more aspects of their fitness and aggressiveness will be examined. Moreover, more than one susceptible cultivars (e.g. Topas) will be included in the experiments. New UK isolates for testing will be obtained before/after harvest in summer 2012 from phoma stem cankers of cultivars carrying the *Rlm7* and *Rlm4* genes.

Keywords: *Leptosphaeria maculans*, *Brassica napus*, R gene-mediated resistance, durability, *AvrLm-Rlm*

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Impact of crop successions including legumes species on the durability of partial resistance to *Aphanomyces euteiches* in pea

Le May C.¹; Onfroy C.²; Moussart A.²; Tivoli B.¹; Andrivon D.¹; Baranger A.¹; Pilet-Nayel M.L.¹

¹INRA, AGROCAMPUS Ouest, UMR IGEPP, 65 rue de Saint Briec, 35042 Rennes, France

²Union Nationale Interprofessionnelle des Plantes riches en Protéines (UNIP), 11 rue de Monceau, 75378 Paris Cedex 08, France

Abstract

The impact of alternative host on plant pathogen adaptation processes must be taken into account for the durable management and of resistance cultivars. Infection of several host plants is a prevalent process in agrosystems, leading to change in epidemic process and pathogenicity. Indeed, this process modifies the survival of pathogen populations, and transmission opportunities to different compartments (wild/cultivated, cultivated/cultivated) (Woolhouse *et al.*, 2001). *Aphanomyces euteiches* Drechsler is an oomycete pathogen that causes devastating root rot of pea (*Pisum sativum* L.) worldwide. This homothallic pathogen is polyphagous and was reported to attack other legume species (Levenfors, 2004; Moussart *et al.*, 2008). Adaptation event of *A. euteiches* to host plant resistance has already been observed in alfalfa, as isolates overcoming genetic resistance to race 1 of *A. euteiches* have been identified (Grau *et al.*, 1991). Whereas sexual recombination and genetic flow remained low for

evolutive processes of *A. euteiches* populations (Grunvald and Hoheisel, 2006), a possible role of host plants in the pathogen population structuration could be suggested. Genetic resistance to *A. euteiches* in pea is partial and controlled by several quantitative loci (Pilet-Nayel *et al.*, 2005; Hamon *et al.*, 2011). In order to remain pea crop in agricultural systems, and maintain partial resistance efficiency in pea cultivars, it is necessary to understand the role of legume species, which will be included in rotations, on the *A. euteiches* population adaptation processes. To address this question, we currently develop a research project which is divided in two main objectives: *i*) to evaluate the “filter” effect of legume species used in the rotation, on *A. euteiches* populations (aggressiveness and virulence change, selection, host spectral change, infection potential), and *ii*) to test the durability of pea resistance QTL combinations and scenarios of *A. euteiches* pea resistance QTL deployment in rotations including cereals (non host) and other host leguminous crops (faba bean, alfalfa, vetch). Successions of agronomic types (resistant and susceptible pea or other legumes wheat-barley-rapeseed) and of legume mono-crops will be tested in controlled conditions. A mixture of French strains phenotypically and genetically well-characterized will be used for inoculation. After one to several crop succession cycles, the resistance levels of pea partially resistant lines as well as the evolution of inoculum potential and strains frequency will be evaluated using pathological and molecular tests. The isolated *A. euteiches* pea-infecting strains will be characterized using host specificity and pathogenicity tests (Moussart *et al.*, 2008), as well as polymorphic SSR markers from *A. euteiches* sequencing data. The impact of the different scenarios of crop successions will be analysed regarding resistance loci content of pea genotypes. QTL combinations allowing to limit pathogen adaptation to pea resistance will be identified.

Keywords: *Oomycota*, legume species, evolutive process, crop rotation

Session 2
Sustainable and integrated
breeding
and
deployment of genetic resistance

VINQUEST: The monitoring of *Venturia inaequalis* virulences initiative

Patocchi A.¹; Auwerkerken A.²; Masny S.³; Nybom H.⁴; Phillion V.⁵; Tamm L.⁶; Vávra R.⁷; Peil A.⁸; Padder B. A.⁹; Rühmer T.¹⁰

¹Agroscope Changins Wädenswil Research Station ACW, 8820 Wädenswil, Switzerland

²Better3fruit, 3202 Rillaar, Belgium

³Research Institute of Horticulture, Pomology Division, Pomologiczna 18, 96-100 Skierniewice, Poland

⁴Balsgård-Department of Plant Breeding and Biotechnology, Swedish University Of Agricultural Sciences, 29194 Kristianstad, Sweden

⁵Institut de recherche et de développement en agro-environnement, J3V 0G7 Saint-Bruno-de-Montarville, Canada

⁶Research institute of organic agriculture (FiBL), 5070 Frick, Switzerland

⁷Research and Breeding Institute of Pomology Ltd., 508 01 Horice, Czech Republic

⁸Julius Kühn-Institut, Federal Research Centre for Cultivated Plants, Institute for Breeding Research on Horticultural and Fruit Crops, D-01326 Dresden, Germany

⁹Division of Plant Pathology, Sher-e-Kashmir University of Agricultural Sciences and Technology of Kashmir, 191 121 Srinagar, Jammu and Kashmir, India

¹⁰Landwirtschaftliches Versuchszentrum, 8047 Graz, Austria

Abstract

Apple scab, caused by *Venturia inaequalis*, is the most important fungal disease of apple in temperate regions. Cultivation of apple cultivars carrying durable scab resistance is one of the solutions proposed to produce this fruit in a sustainable way. Durable, or at least more durable, resistance can be achieved by pyramiding resistance genes into one genotype. But what genes should be used in these pyramids? Such information can only be obtained with a global evaluation of the resistance genes for the corresponding virulences in the pathogen populations. An international initiative was recently taken to collect this information in a standardized way through the monitoring of virulences in different *V. inaequalis* populations. The results of the initiative are made available to the breeders yearly through the homepage www.vinquest.ch (Patocchi et al. 2009).

Since its start in 2009, 29 partners in 16 countries have joined the initiative. All of them have established or are in the process of establishing trap orchards comprising the same 16 differential hosts. The typical trap orchard consists of 5 randomized blocks with one-tree plots of each host h(0) to h(15) (80 plants in total), and 5 placeholders (generally susceptible cultivars) per block that will be substituted in the future with genotypes carrying new scab resistance genes (105 plants in total). To facilitate the export of the material outside of Europe the establishment of *in vitro* cultures of the differential hosts has been initiated.

An overview of the initiative as well as the first results obtained will be presented.

Keywords: apple scab, durable resistance, apple breeding

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Importance of the genetic background for sustainable resistance: experimental evidence for a major resistance gene to nematodes

Barbary A.¹; Palloix A.²; Fazari A.¹; Marteu N.¹; Castagnone-Sereno P.¹; Djian-Caporalino C.¹

¹INRA PACA (Provence Alpes Côte d'Azur), UMR1355 INRA/UNSA/CNRS, Institut Sophia Agrobiotech, BP167, F-06903 Sophia Antipolis, France

²INRA PACA, UR1052, Génétique et Amélioration des Fruits et Légumes, F-84143 Montfavet, France

Abstract

Root-knot nematodes, *Meloidogyne* spp., are extremely polyphagous plant parasites worldwide. Since the use of most chemical nematicides is being prohibited, genetic resistance is an efficient alternative way to protect crops against these pests. However, resistance genes (R-genes) are limited and nematode populations are able to overcome them with time. Good management of these valuable resources is thus a key point of R-gene durability. In pepper, *Me3* is a dominant major resistance gene, currently used in breeding programs, that control *M. arenaria*, *M. incognita* and *M. javanica*, the three main root-knot nematodes species. In this study, it was introgressed in either a susceptible or a partially resistant genetic background in either homozygous or heterozygous allelic status. Confronting these genotypes with a high inoculation pressure of an avirulent *M. incognita* isolate or a *Me3* virulent laboratory-selected population (obtained by successive re-inoculation on a *Me3* R-pepper line) demonstrated i) that the genetic background plays an important role, *Me3* being overcome more easily in a susceptible genetic background than in a partially resistant one, ii) that the allelic status has no effect. These results are in good agreement with concepts recently developed from the analysis of very different plant-pathogen interactions: pepper-virus (Palloix et al., 2009) or rapeseed-*Leptosphaeria* (Brun et al., 2012). Experiments are now underway to detect and localise genes or loci providing partial resistance (QTLs = Quantitative Trait Loci) to root-knot nematodes explaining the differences observed between susceptible and partially resistant genetic backgrounds, and to determine the effectiveness of their « protective » role on the major R-genes. All these results are of main importance for the creation of new varieties by breeders who have to take into account the plant material used and the resistance gene they want to introgress.

Keywords: *Meloidogyne* spp., *Capsicum annuum* (pepper), Me(s) resistance genes, dosage allele effect, resistance durability

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Differentiation in the susceptibility of German winter wheat cultivars against *Mycosphaerella graminicola* isolates under controlled and field conditions

Rodemann B.

Institute for Plant Protection in Field Crops and Grassland, Julius Kühn-Institut; Braunschweig, Germany

Abstract

Mycosphaerella graminicola (*Septoria tritici*) is the most damaging foliar disease in Europe in wheat causing significant yield loss every year. By intensive cultivation techniques, an increasing proportion of wheat in the crop rotation and a varying range of varieties have increased the importance of leaf blight (Orton et al.; 2011). In extreme cases, yield losses were observed up to 30%, mainly through a reduction of thousand kernel weight. Yield losses resulting from the loss of green leaf area, leading to a retention of assimilates and nitrogen compounds in the leaves.

The infection risk can plant growing through plowing of infested straw and stubble residues and reduce a later sowing. These low susceptible varieties should be planted that, if possible should not be extremely short to prevent the transport of high pycnospores. In addition, the high incidence of disease could be stopped applying fungicides early. Given the long latency period, combinations with curative and protective active ingredients are sprayed as close to the time of infection. For choosing the most effective fungicide, the problem of the increased incidence of *Septoria* resistance against strobilurins and are considered less sensitive biotypes compared with azoles, must be considered (Thygesen et al., 2009). As a control threshold for the initial treatment in 50% of infected plants is BBCH 32 or precipitation of more than 3 mm and more than 48 hours of leaf wetness > 98%.

The objective of the present study was determining the levels of resistance against *M. graminicola* of 64 different winter wheat cultivars. Methodological studies in the aggressiveness of *M. graminicola* isolates were tested on 8 winter wheat varieties. In field trials with artificial inoculation the susceptibility of 64 German varieties was evaluated. In the development stage BBCH 39 to BBCH 49 the plants were inoculated with a *M. graminicola* conidial suspension of 1×10^6 conidia / ml. The infestation on flag leaf, the first and second leaf was assessed on a scale from 0 to 100% determined.

In the greenhouse studies, the infestation 21 days after inoculation moved between 3% and 25%. For the varieties Tabasco and Jenga the best tolerance against several *M. graminicola* isolates were determined. In the field, an infestation was found from 16% to 89% approximately 35 days after inoculation. The lowest susceptibility was determined for the varieties of Tabasco, and Lear, Julius.

Keywords: *Mycosphaerella graminicola*, necrotroph, leaf spot, winter wheat, cultivar resistance

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Effects of lettuce cultivar mixtures, differing for resistance genes, on *Bremia lactucae* incidence

Maisonneuve B.¹; Martin E.¹; Jean L.¹; de Vallavieille-Pope C.²; Pitrat M.¹

¹INRA-UR1052, Unité de Génétique et d'Amélioration des Fruits et Légumes, Domaine Saint Maurice, BP94, 84143 Montfavet Cedex,

²INRA, UR1290 BIOGER-CPP, BP01, 78850 Thiverval Grignon, France

Abstract

Bremia lactucae, the causal agent of lettuce downy mildew, is still an important problem in lettuce production in spite of many resistance genes used over the last 50 years in Europe. Every new gene introduced into commercial varieties has been overcome in a few years. At INRA, new resistance genes were found in two *L. virosa* accessions and introgressed into butterhead lettuce (lines named Vi). These resistance genes are not yet introduced in commercial varieties; we would like propose a using strategy of these genes that could increase their durability.

Which strategies might be efficient to reduce the evolution of the pathogen and ensure a durable resistance with these genes? A lower selection pressure on *B. lactucae* population could reduce the speed of breakdown of the new resistance. A possibility to have a lower selection pressure on the *B. lactucae* populations could be mixture of two cultivars differing for resistance genes. Therefore *Bremia* contamination has been studied for several winters in cultures of these resistant Vi lines mixed with a susceptible cultivar. In the same culture, the progression of *Bremia* on the susceptible plants has been observed. The disease development has been studied after an artificial contamination with two objectives: (i) does the pathogen overcome the resistance, (ii) is the disease severity on the susceptible cultivar acceptable for commercial use?

After eight experimentations under a polythene greenhouse, pure stands were compared to mixture in ratio 1:3 (susceptible:resistant) after focal inoculation with a *B. lactucae* isolate common in the region. No sporulation has been observed on the two Vi lines issued from *L. virosa*.

For the year with high inoculum pressure, the cultivar mixture did not reduce the disease severity on the susceptible cultivar (for example, in winter 2006-2007, 24 contaminated leaves per susceptible plant in pure stand and in mixture; a disease severity of 53 and 48 on the susceptible variety, respectively in pure stand and in mixture).

For intermediate to low inoculum pressure, the disease development was slower on the susceptible cultivar grown in mixtures, with less contaminated plants and less contaminated leaves per plant (1.1 in pure stand vs 0.2 in mixture, in winter 2005-2006) at harvest. The disease severity on susceptible cultivar is lower in mixture than in pure stand for example in winter 2005-2006 (index 1.3 vs 9.5); this severity was similar at the beginning of the epidemics for both treatments, but it became much higher in pure susceptible stand on the last week before harvest.

Therefore the mixed culture could be an interesting method to reduce disease level on a cultivar susceptible to the strain that is important in the culture and also possibly increase the durability of new resistance by a lower selection pressure on the virulent strains. Nevertheless this cultural practice must be adapted to the needs of growers economically and technically; it could be difficult to have an efficient design of the mixture in a commercial culture. Some other experiments must be done in connection with the growers and the breeders.

Keywords: lettuce, *Lactuca*, *Bremia lactucae*, resistance, cultivar mixture, epidemiology

The GEDUNEM project: Varietal and technical innovations for the sustainable and integrated management of root-knot nematodes in protected vegetable cropping systems

Djian-Caporalino C.¹; Palloix A.²; Navarrete M.³; Lefevre A.⁴; Mateille T.⁵; Védie H.⁶; Goillon C.⁷; Trottin Y.⁸; Boniol P.⁹; Castagnone-Sereno P.¹

¹INRA PACA (Provence Alpes Côte d'Azur), UMR1355 INRA/UNSA/CNRS, Institut Sophia Agrobiotech, BP167, F-06903 Sophia Antipolis, France

²INRA PACA, UR1052, Génétique et Amélioration des Fruits et Légumes, F-84143 Montfavet, France

³INRA PACA, UR0767, Écodéveloppement, Domaine Saint-Paul, Site Agroparc F- 84914 Avignon Cedex 9, France

⁴INRA, Sciences pour l'Action et le Développement, Domaine Expérimental de Alénia Roussillon, Mas Blanc, 66200 Alénia, France

⁵IRD, UMR CBGP, Campus de Baillarguet, CS30016, F-34988 Montferrier-sur-Lez Cedex, France

⁶GRAB, Groupe de Recherche en Agriculture Biologique, BP 11283, 84 911 Avignon Cedex 9, France

⁷APREL, Association Provençale de Recherche et d'Expérimentation Légumière, Route de Mollégès, 13210 Saint-Rémy de Provence, France

⁸CTIFL, Centre Technique Interprofessionnel des Fruits et Légumes, Centre de Balandran, BP 32, 30127 Bellegarde, France

⁹Groupe Azura, Disma International - sales office, 332, rue de Turin, BP 5434, 66034 Perpignan Cedex, France & Société Maraissa, Route de Tiznit, km 39 Tin mansour, Chtouka Aït Baha, Agadir, Maroc.

Abstract

The recent banning of the most active chemical nematicides raised root-knot nematodes (RKN) as the major problem of vegetable growing in the south-east of France, with over 40% of horticultural farms affected. Resistant (*R*) cultivars successfully limit nematode attacks of the crop itself and reduce nematode abundance in soil in the short term. But their use in commercial fields faces two major constraints: (1) the limited number of cultivated species with RKN *R*-genes available (tomato, pepper), which would lead to major changes in crop rotations with socio-economic constraints; (2) the emergence of virulent root-knot nematode populations, able to overcome the resistance conferred by some of the *R*-genes. Recent research showed that the durability of the resistance per se was possibly increased with an optimal choice of the *R*-gene or allele combination, and of the genetic background in which the major *R*-genes were introgressed. However, the requirement for agronomic performance, does not always allow the breeder to fully use the genetic diversity available in *R*-genes and genetic backgrounds. In such cases, spatio-temporal deployment strategies of resistant cultivars in the rotation were proposed and provided some success. But combination of genetic resistance with cultivation practices including multicrop rotations, intercultural management and/or prophylactic treatments was poorly tested for its ability to provide complementary selection pressures on the pathogen populations and to increase the durability of the protection.

The project aims at identifying innovative strategies across the agrosystem, combining varietal resistance in crop rotations with agronomic practices, for a sustainable control of RKN that infect vegetable crops in protected crop systems. This pilot and demonstrative project involves analytical and systemic aspects: i/ the validation of previous results on the durability of RKN genetic resistance in tomato and pepper by long-term experiments in research stations and in farms, ii/ the impact of agronomic practices on the parasitic pressure in the soil and its contribution in the increase of resistance durability, and iii/ the impact of such innovations on the yield and the economic viability for protected

crop systems in a Mediterranean climate. The added value here is to combine together currently dispersed approaches based on synergistic and long-term effects, towards a satisfying level of nematode control over pluri-annual crop sequences. The diversity of partners (research, experimental stations, technical institutes) and associated forces (growers' development structures, 'chambres d'agriculture') brings all the complementary expertise needed for answering specific short-term questions as well as generic mid-long term expectations.

'GEDUNEM' has been launched in the framework of the INRA metaprogramme SMaCH (Sustainable Management of Crop Health).

Keywords: integrated pest management, root-knot nematodes, crop rotation in vegetable farming, genetic resistance to nematodes, agronomic practices

Genetic (QTL) mapping approach revealed unexpected patterns of bypassed and sustainable resistances to *Microcyclus ulei* in rubber tree (*Hevea spp.*).

Seguin M.¹; Garcia D.¹; Mattos C.²; Doaré F.³; Le Guen V.¹

¹Cirad, UMR-AGAP, Montpellier (France)

²Michelin, PMB, Ituberà (Bahia, Brazil)

³Cirad, UR-106, Kourou (French Guiana)

Abstract

The South American Leaf Blight (SALB), due to the Ascomycota *Microcyclus ulei*, threatens the world production of natural rubber, based on the cultivation of nearly only one species: the tropical tree *Hevea brasiliensis*. In connection with a breeding program, conducted in Brazil by Michelin and Cirad and aiming to create resistant cultivars of rubber tree, we analysed the genetic determinism of 3 sources of natural resistance to SALB. We developed a classical approach of QTL mapping on 5 segregating populations issued from crosses implying 3 resistant *Hevea* accessions, showing contrasting type of resistance: partial or complete, bypassed or sustainable. Disease resistance of the progenies was assessed under natural infestation in field trials, in Brazil and French Guyana, or under controlled inoculations with isolated strains of *Microcyclus ulei*.

Genetic mapping revealed unexpected genetic determinisms underlying the observed resistances: whereas a complex genetic determinism implying major resistance genes and several minor QTLs can be bypassed by the pathogen (Le Guen *et al.* 2007), a durable partial resistance appeared to be governed by only 2 major resistance loci (Le Guen *et al.* 2011). These original results constitute a favorable context for a marker-aided selection of cultivars combining high latex yield and SALB tolerance.

Keywords: Rubber tree; *Hevea brasiliensis*; *Microcyclus ulei*; Genetic determinism; Genetic mapping

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Combining polygenic resistance with prophylactic and cultural methods for sustainable management of *Aphanomyces* root rot in pea

Pilet-Nayel M-L.¹; Hamon C.²; Lesné A.^{1,3}; Le May C.⁴; Baranger A.¹; Tivoli B.¹; Moussart A.^{1,3}

¹INRA, UMR IGEPP, Domaine de la Motte, 35653 Le Rheu Cedex, France

²Vegenov-BBV, Penn ar Prat, 29250 Saint Pol de Léon, France

³UNIP, 11 rue de Monceau, 75378 Paris Cedex 08, France

⁴Agrocampus-Ouest, UMR IGEPP, 65 rue de Saint Briec, 35042 Rennes, France

Abstract

The combined use of polygenic resistance and alternative methods to control plant diseases could be a choice strategy to preserve efficiency of genetic resistances, by maintaining both low probability of occurrence of multiple mutations from the pathogen for virulence and low size of pathogen populations over time. This strategy is under investigation for managing *Aphanomyces* root rot, one of the most damaging diseases of pea worldwide, against which no efficient method of control is currently available.

Polygenic partial resistance to *Aphanomyces euteiches* has been studied for over ten years but resistant varieties are not available yet. From four RIL populations derived from bi-parental crosses involving main pea sources of resistance available, a total of 27 genomic regions associated with partial resistance were identified, including 7 regions highly consistently detected over populations, environments and strains studied (Hamon, 2011). The seven QTL were introgressed, individually or in various combinations, in elite genetic backgrounds, resulting in Near-Isogenic Lines (QTL-NILs), which were evaluated for their levels of resistance. Furthermore, assays conducted under field conditions for combining polygenic resistance present in breeding lines and prophylactic methods have indicated that growing partial resistant genotypes in soils with low infestation levels resulted in decreasing inoculum potential over time. Cultural methods have also been suggested of interest to be combined with polygenic resistance. Especially, the disease evolution and its impact on yield have been shown to be lower on Winter-sown peas than on Spring-sown peas, due to a partial escape of Winter peas to favorable conditions for disease development. The choice of non-host or resistant legume species in rotations has also been reported as another method to decrease soil inoculum potential (Moussart et al., accepted) and will be studied for its effect on evolution of pathogen population structuration (Le May et al. 2012).

Consequently, the QTL-NILs developed for resistance to *A. euteiches* will be used for studying action modes of resistance QTL, in combination or not with the use of prophylactic and cultural practices, on *A. euteiches* population size and structure. Work is in progress for identifying combinations of *Aphanomyces* root rot methods of control, aiming at preserving polygenic resistance efficiency in pea.

Keywords : *Pisum sativum*, *Aphanomyces euteiches*, QTL, legumes, inoculum potential

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The characteristics of resistance to *Fusarium* crown rot in wheat

Sutherland M. W.; Knight N. L.; Martin A.

Abstract

Crown rot of wheat, caused by several fungal species in the *Fusarium* genus, is a major cereal disease in many of the world's production regions, particularly when seasons are characterised by post-anthesis water stress. In Australia, the most significant crown rot pathogen is *Fusarium pseudograminearum*. Economic losses due to this disease have risen in the past two decades and are now estimated to average \$AUS 79 million per year (1). This increase is partly due to the adoption of minimum tillage practices which retain infected standing stubble between growing seasons. Screening for resistance to this disease in the field is challenging, with disease expression being significantly impacted by genotype by environment interactions and inoculum distribution, which is in turn affected by cropping history. Nevertheless sources of partial resistance to the pathogen have been identified which appear to be effective across growing environments on several continents. These sources include the hexaploid bread wheat lines Sunco, 2-49, IRN 497, CPI133814 and W21MMT70. Analysis of doubled haploid wheat populations incorporating independent sources of resistance, at both the seedling and adult plant stage, reveal multiple and additive quantitative trait loci of relatively small individual effect. To date the mechanisms of resistance are uncharacterised and limited gene expression analysis has been inconclusive. Screening of fungal isolates across a differential set of wheat lines indicates significant variation in aggressiveness, but a lack of distinct pathotypes. Comparisons of the location of visible disease symptoms with pathogen distribution in the tissue, revealed by quantitative PCR, indicate a close association and no evidence of symptomless infection, irrespective of host genotype. Fluorescence microscopy of disease progression in seedling and adult tissues shows that from the time of initial infection, growth of the pathogen in partially resistant tissues is significantly slower than in very susceptible genotypes, but without evidence of the construction of enhanced physical barriers that might impede fungal progress. Resistance levels equivalent to those observed in wheat have not been identified in tetraploid durum materials, which are highly susceptible. Recently, we have successfully transferred partial resistance from several hexaploid wheats into tetraploid durum wheats, demonstrating the utility and robust expression of the hexaploid resistance in diverse genetic backgrounds, despite its quantitative nature.

Keywords: *Fusarium pseudograminearum*; marker assisted selection; durum wheat; quantitative resistance

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Use of genes for resistance to powdery mildew of tomato from various sources in order to create genotypes with durable resistance

Tereshonkova T.A.^{1,2}; Gorshkova N.S.^{1,2}; Emelina, M.N.¹; Khovrin A.N.^{1,2}; Ignatova S.I.¹

¹Russian Research Institute of Vegetables, bld.500, Vereya, Ramensky dst., Moscow region, Russia 140153

²Agrofirma Poisk-vegetable and flower seeds, bld.501, Vereya, Ramensky dst., Moscow region, Russia 140153

Abstract

Since the 90s powdery mildew (*Oidium neolycopersici*) has been economically significant disease of the greenhouse tomato culture in Russia. The reports of appearance of a new race, which overcame resistance gene *Ol-1* (Kashimoto et al., 2003), as well as genetic differences of geographical isolates of *O. neolycopersici* (Kiss L. et al., 2005) and our own research on differences between isolates from China and from two region of Russia indicates the presence of variability in the pathogen population. As tomato resistance to *O. neolycopersici* is controlled by several dominant and recessive genes, as well as several QTLs (Li Chengwei et al., 2011), the resistance based on any one of them may be overcome. We selected five sources of resistance of cherry tomatoes, derived from interspecific crosses with wild species such as *Solanum pimpinellifolium* L., *Solanum habrochaites* S. Knapp & D.M Spooner (*S. hirsutum*), *S. hirsutum* v. *glabratum*, which characterized by varying levels of resistance to the disease from almost immune to the high degree of resistance with symptoms of chlorosis and poor sporulation. Donors of resistance were obtained by recombination and selection under conditions of the artificial inoculation, so we can see the genome does not have potentially dangerous alien genes. Hybrids obtained by diallel crossing scheme between the five sources of resistance and susceptible genotypes show a different levels of resistance that has allowed to conclude that the character of trait inheritance received from various sources. Also we have obtained a mildew-resistant genotypes possessing a set of good agronomic traits. During the years of research resistant genotypes did not lose its resistance, indicating the stability of the pathogen. An interesting and still not finding an explanation is the fact that the most stable and valuable donor of resistance, (F9-F12) derived from the crosses between *S. habrochaites*, *S. pimpinellifolium* *S. lycopersicum* in crosses with susceptible genotypes as male form gives 4 resistant hybrids out of 5, but as a female parent - 3 out of 5. Probably the reason of this fact is an interfere of genome of a second component of crossing or the phenomena of segregation in intercrossing genotypes.

Keywords: tomato, *Oidium neolycopersici*, levels of resistance

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Comparison of winter wheat and triticale cultivars in the seedling stage and adult plant stages to *Stagonospora nodorum*

Ziemichod M.E.; Arseniuk E.

Plant Breeding and Acclimatization Institute –National Research Institute, Radzikow,05-870 Blonie, Poland

Abstract

Stagonospora nodorum is a fungus that causes glume and leaf blotch of cereal small grain species. Destruction of cereals by necrotrophic fungi of the *Stagonospora nodorum* and *stagonospora spp.* is recorded in Poland every year with varying intensity. The severity of *Stagonospora nodorum* blotch (SNB) disease is also modified each year by weather conditions. This fungus infects all of assimilating organs of the plant. Foster the development of moderate temperatures and high humidity.

The aim of the study was to compare the response to SNB varieties of winter triticale and winter wheat seedlings at second leaf and adult plant growth stages. Plants were subjected to phenotypic evaluation, taking into account the SNB severity on leaves.

The first step was to conduct tests with seedlings under controlled environment for disease severity on seedlings of 13 varieties of winter triticale and 29 varieties of winter wheat.

Seedlings were inoculated with an aqueous suspension of pycnidiospores of 15 *S. nodorum* isolates at a concentration of 12 million spores per ml. Two weeks after inoculation an evaluation was performed to assess the extent of second seedling leaf infection by *S. nodorum* on a gradual scale of 9 digits (1^o - resistant, 9^o - susceptible). Disease symptoms ranged, respectively for wheat cultivars from 4.0 to 9.0 and for triticale cultivars from 4.0 to 6.6 on the scale. Among triticale cultivars the most susceptible appeared to be Algosó and among wheat ones Mulan and Smagard.

The second step was to examine varietal responses to SNB in the field. SNB severity was rated on mature leaves of winter triticale and winter wheat varieties. The same sets of varieties were used at both growth stages. Each variety was planted to 1m² plot. During the growing season disease severity was rated on leaves and ears of varieties tested.

The experiment was performed in duplicate in a randomized block layout. Plants on plots were inoculated for three times with an aqueous suspension of *S. nodorum* pycnidiospores at a concentration of 6 million spores per ml. The first inoculation was carried out in the early boot stage (GS 45 according to the scale of Zadoks), a second inoculation was performed at the stage of heading, and a third one at the early flowering stage (GS 59). Control plots were protected with Tilt 250 EC (0.1% sa - propiconazole). Parameter evaluated in the experiment was disease severity, also on 9 digit scale, from 1 to 9 (where 1 means leaf susceptibility, 9 – resistance). Both experiments were elaborated statistically to highlight the most and least susceptible cereal cultivars.

The scope of reaction of leaves of mature plants seen in the field ranged, respectively for varieties triticale from 3.9 (Algosó) to 5.7 (Borwo) and wheat from 4.6 (Bagou) to 6.3 (Jengi) on 9^o scale.

Significant differences in resistance to *S. nodorum* were found among tested cultivars of both cereal species. Coefficients calculated for both stages of cereal cultivars were as follows: for triticale Spearman correlation $r_{\text{Spearman}} = 0.214$ and determination coefficient $R^2 = 0.0456$. This means that the resistance of the studied group of plant leaves triticale cultivars was determined in only 4,6 % by the resistance of seedlings during the second leaf. On the other hand only cultivar Algosó was ranked identically at 13 position at both growth stages. It was also the most susceptible at both growing stages. Other triticale cultivars differed significantly in their rankings on SNB level.

Coefficients of correlation for compared growth stages for wheat cultivars were: Spearman rank coefficient $r_{\text{Spearman}} = 0.417$ and determination coefficient $R^2 = 0.174$.

This means that the resistance of the leaves of plants tested group of varieties of wheat was determined

in 17.4% of the resistance of seedling leaf resistance among the cultivars tested solely three of them, i.e. Jenga, Kranich i Askalon showed quite consistent ranking at both compared stages. All other varieties differed significantly in their ranks and thus resistance to *Stagonospora nodorum*. Comparing the resistance of cereal species tested should be noted that a group of cultivars of triticale on balance proved to be more vulnerable than test group of varieties of wheat. In conclusion also be noted that for the tested group of varieties of cereals in the case of wheat although coefficient of determination was higher but not so high to test for resistance to *Stagonospora nodorum* limited only to test seedlings.

Effect of climate conditions and frost damage on the occurrence of grey mould disease caused by *Botrytis cinerea* in oilseed rape fields

Zamani-Noor N.; Rodemann B.

Institute for Plant Protection in Field Crops and Grassland, Julius Kühn Institute; Brunswick, Germany

Abstract

Botrytis cinerea is a non-specific necrotrophic pathogen that causes grey mould disease in a broad range of plant species worldwide. Previous studies have shown that botrytis infection of oilseed rape (*Brassica napus*) occurs conspicuously late in the growing season on dead and dying plant parts and crop debris. Mild and humid weather conditions favour the disease, and the pathogen can initiate infection on leaves, stems, flowers and pods (Lane and Gladders, 2008; Oxley and Evans, 2009). Furthermore, it was shown that the fungus can also attack the green living tissue via holes and cracks caused by frost, cabbage seed weevil or fertilizer instruments (Barnes and Shaw, 2002; Oxley and Evans, 2009). Little is known about the severity of damage and the level of yield losses of oilseed rape crops caused by *B. cinerea*. In 2012, in some regions of Germany, oilseed rape fields were affected by a late March frost during a period of low night temperatures. Following these low temperatures, a high incidence of grey mould infection developed on frost-damaged plant tissues. Typical disease symptoms of distinct mouldy lesion on hypocotyls covered by abundant grey mycelia, conidiophores and spores were detected. Within a few days, the lesion continues to expand and the disease became much more intense until severely infected plants collapsed and died. The objective of the present study was determining the levels of resistance against *B. cinerea* of 80 different oilseed rape cultivars. Levels of disease incidence and disease severity were assessed using a scale of 0–4, where 0 = no symptoms and 4 = dead plants. Results revealed that all cultivars of oilseed rape were susceptible to the pathogen, but they displayed significant differences in their response to botrytis infection. While the mean disease incidence was between 81.3% and 100%; disease severity ranged from 41.3% to 73.0%. We concluded that grey mould disease can become an important disease in oilseed rape fields under extreme environmental conditions, and that a severe infection can result in a high incidence of plant death.

Keywords: necrotroph, *Brassica napus*, hypocotyls, low temperature

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Brown rot fungus *Monilia fructigena* in the centre of apple origin Aitkhozhina N.

Institute of Microbiology and Virology, 103 Bogenbai batyr str., Almaty, Kazakhstan

Abstract

The apple (*Malus domestica* L.) is considered to have its origin in mountain area of south-east Kazakhstan and eastern part of China on the border with Kazakhstan. Silky way traders introduced the fruit into Persia and later Balkans and almost all Europe. South-east Kazakhstan has a valuable apple-producing tree. Until early ninetieth of last century no brown rot has been recorded in this area. Natural resistance to brown rot is found in a small restricted area of cultivated apples in south-east Kazakhstan and in a small wild orchard in a high mountain zone. Nowadays almost all commercial and private orchards except wild trees are heavily infested with *M.fructigena*. Garden pear (*Pyrus communis* L.), the peach (*Prunus persica* L.), the apricot (*P.armenica* L.), the quince (*Cydonia oblongata* L.) are also under the pathogen. During our studies we couldn't isolate a great diversity of brown rot fungi. The main pathogen was commonly constituted by variants of *M.fructigena*. They were characterized by different conidia size, sporodochia pigmentation, mummies and absence of perfect stage. One strain of *M.laxa* was also found in microfungus isolates. No blossom or twig blight in all apple growing areas were happened. *M. fructigena* attacks small fruits like gooseberry (*Grossularia setosa* L.) and black currant (*Ribes nigrum* L.). Intensive cultural practices and introduction of new cultivars from Ukraine, Russia and China favoured of epiphytotic outbreaks. Subspecies of *M. fructigena* appears constantly and they have a high level of pathogenicity. Fortunately, *Monilinia fructicola* haven't yet appeared in Kazakhstan. Understanding of the evolution of brown rot pathogens in the centre of apple origin will give a profuse information towards the evolution of fruit trees and brown rot fungi, and their coevolution.

Keywords: apple, origin, brown rot fungi, pathogenecity, coevolution

Population fluctuations, relative susceptibility and control of *Thrips tabaci* (Lind.) on some onion and garlic cultivars and strains

Amro M.A.¹; Abd El-Rahim G.H.²; Abd El-Raheem A.A.¹

¹Plant Protection Research Institute, Agricultural Research Center, Dokki, Giza moamro1953@yahoo.com

²Horticulture Department, Faculty of Agriculture, El-Azhar University

Abstract

Bulb characters of two onion cultivars and two strains were determined. The total yield was arranged in descending order as follows: Al-Azhar noua by 15.62 > Giza 20 by 14.16 > El-Baraka noua by 13.48 > Giza 6 Mohassan by 13.06 ton/feddan. Exportable yield and culls yield were also determined. Population fluctuations of *Thrips tabaci* (Lind.) and resistance status of the tested onion and garlic cultivars and strains to this insect pest were determined in more details. In respect to the pest incidence, no significant difference was obtained between the tested onion cultivars or strains. However, significant difference was obtained between the inspection dates. The pest incidence on onion during the entire period of study showed that Giza 6 Mohassan harbored 25.60, Al-Azhar noua 23.55, Giza 20 22.86 and El-Baraka noua 22.09 individuals/plant. Consequently, Giza 6 Mohassan and Al-Azhar noua appeared as susceptible (S) cultivars, while Giza 20 and El-Baraka noua showed some sort of resistance and appeared as low resistant (LR) cultivars. Local garlic harbored 1.53 times of the pest more than Chinese cultivar. Consequently, the local garlic appeared as susceptible (S) cultivar, while the Chinese garlic appeared as moderately resistant (MR) cultivar. The ability of chemical and safe alternative insecticides in reducing the pest infestation on onion plantations arranged in descending order as follows: Spinosad by 85.67% > Sumithion by 80.89% > Achook by 64.05% > Kemsol oil by 48.25%. Application of the tested compounds increased the yield by 21% more than untreated plants.

Keywords: *Thrips tabaci* (Lind.), onion, garlic, resistance status, control

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Biological features, positional cloning and validation of the *Ma* gene for high-level and complete-spectrum resistance to root-knot-nematodes in *Prunus*

Van Ghelder C.¹; Khallouk S.¹; Polidori J.¹; Duval H.²; Kleinhentz M.³; Dirlewanger E.³; and Esmenjaud D.¹

¹INRA, UMR ISA, INRA1355, CNRS7254, Université de Nice, F-06903 Sophia Antipolis, France; daniel.esmenjaud@sophia.inra.fr

²INRA, UR Génétique et Amélioration des Fruits et Légumes (UGAFL), F-84914 Avignon, France

³INRA, UR Espèces Fruitières (UREF), F-33883 Villenave d'Ornon, France

Abstract

Stone fruit crops *Prunus* spp. grown under Mediterranean climates are severely damaged by root-knot nematodes (RKN) *Meloidogyne* spp. and breeding for RKN-resistant rootstocks is a promising control alternative to nematicide ban. Resistance (R) genes that confer different spectra have been identified and mapped in plums (*Ma* and *Rjap*), peach (*RMia*) and almond (*RMja*). Thus sustainable resistance in *Prunus* is based on pyramiding of R genes in interspecific rootstock material to take into account the risk of resistance breaking in the durable plant-nematode interaction for perennials. The *Ma* gene from Myrobalan plum has been shown to confer a complete-spectrum, high-level and heat-stable resistance to both mitotic (*M. arenaria*, *M. incognita*, *M. javanica* and *M. enterolobii*) and meiotic (*M. floridensis*) RKN. *Ma* triggers a hypersensitive-like reaction (HLR) in root apices and severe nematode attacks induce the development of subterminal lateral rootlets replacing primary terminal apices and providing an active resistance response to HLR damage (1). Sustainability of resistance conferred by *Ma* has been challenged in multi-year experiments applying a high and continuous nematode inoculum pressure by co-cultivation of RKN-infested susceptible tomato plants together with *Prunus* plants carrying *Ma* or, for comparison, with R tomato plants carrying the *Mi-1* reference gene. Galling and virulent nematode individuals have been observed in *Mi-1* resistant tomatoes but not in *Ma*-carrying *Prunus* plants. The positional cloning of the *Ma* locus in accession P.2175 has been performed using high resolution mapping developed in two successive steps totalling over 3000 segregants. The *Ma* locus interval has been reduced to a 32-kb cluster of three TIR-NB-LRR genes (TNL1 to TNL3) including a pseudogene (TNL2) and a truncated gene (TNL3). Using *A. rhizogenes* transformed hairy roots and composite plants (2), the best candidate gene, TNL1, comprising the genomic sequence and the native promoter region (15.3 kb), has been validated as *Ma* as it conferred the same complete-spectrum and high-level resistance as in the donor accession P.2175. The full-length cDNA (2048 aa) of *Ma* is the longest of all R genes cloned to-date. Its TNL structure is extended by a huge C-terminal post-LRR (PL) region (1088 aa) comprising five repeated PL exons (3).

Keywords: *Meloidogyne*, plant resistance, *Prunus*, root-knot nematode, sustainable resistance

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Simulation of race dynamics assists durability of blast resistance in multilines in Japan

Ashizawa T.¹; Nakabayashi J.²; Sasaki A.³; Ishikawa K.⁴; Sasahara M.⁵; Tsukamoto S.⁶; Koga H.⁷; Watanabe T.⁸; Hirayae K.⁹

¹NARO National Agriculture and Food Research Organization, Agricultural Research Center

²Tokyo University

³Graduate University for Advanced Studies

⁴Niigata Agricultural Research Institute

⁵Miyagi Prefectural Furukawa Agricultural Experiment Station

⁶Ishikawa Agricultural Research Center

⁷Ishikawa Prefectural University

⁸Fukui Agricultural experiment Station

⁹NARO National Agriculture and Food Research Organization, Kyusyu Okinawa Agricultural Research Center

Abstract

Rice blast caused by *Magnaporthe grisea* is the most serious rice disease worldwide. To control the disease, monogenic blast-resistant cultivars were introduced into cultivation in the 1960s. However, within a few years, the cultivars' resistance was broken down because of increased virulence of blast fungus races.

Development of multiline cultivars, a mixture of monogenic resistance lines, is an effective approach to prevent this breakdown. In Japan, *Sasanishiki BL*, a mixture of several near-isogenic lines (*NILs*), was first introduced into cultivation in 1995 in Miyagi prefecture. However, no tools with which to forecast blast race changes in fields planted in *Sasanishiki BL* were available at that time. For sustainable use of multilines, long-term simulation of pathogen race dynamics in multilines is needed.

Based on a co-evolutionary system between rice resistance and blast race, we developed a race-dynamics simulation model for a multiline. In the simulation, values are set for the following parameters: combination of *NILs* and blast race, changing *NIL* composition in any given year, overwintering probability of the races, meteorological variables, virulence cost, mutation rate, lesion number, and number of calculation trials. The simulation results indicate annual changes in race frequency with a multiline. These simulation data enable decisions to be made on the number of *NILs*, proportions of *NILs*, and timing of changes in the composition of *NILs* before or after introduction of a multiline.

To forecast emergence and increase in frequency of a super-race that would overcome the resistance of all *NILs* in a multiline, the race dynamics were simulated in three representative *NIL* mixtures: i) a pure stand of one *NIL*, ii) a mixture of five *NILs*, and iii) a mixture of seven *NILs*. The results for the respective mixtures indicated that i) a monogenic resistant *NIL* was immediately overcome by the super-race, ii) a multiple-virulent race emerged and became a super-race within a decade, and iii) partially virulent races predominated but did not emerge as a super-race after 100 years. In these simulations, a larger number of component *NILs* in a multiline apparently suppressed the emergence of a newly virulent race at a given mutation rate (10^{-5}).

A large-scale trial was initiated in Niigata prefecture in 2005. The elite cultivar *Koshihikari* was converted into a multiline, *Koshihikari BL*, and planted in a 100,000 ha paddy field. However, the composition of the multiline has been changed only every few years, because sources of resistant *NILs* are limited. Using our simulator, the *NIL* composition in *Koshihikari BL* was readily determined. To prevent breakdown of the resistant *NILs* in *Koshihikari BL*, monitoring of races in the *Koshihikari BL* field has been ongoing by prefectural researchers, who have determined schedules for changes in the composition of *NIL*. This approach represents human- and simulation-supported durability of a multiline. In contrast, *Sasanishiki BL* composed of all eight *NILs* have been planted in a 600 ha paddy field since 2010, because the simulation results clearly indicated the durability of *Sasanishiki BL*. Cultivation of multilines has contributed to a decrease in chemical fungicide application and thus increased food safety for consumers, and conservation-oriented agriculture.

Keywords: multiline, rice blast, resistance, simulation, virulence

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Session 3
From plant-pathogen
molecular interactions
to the durability of resistance

Management of the grapevine fanleaf degeneration: are innovative and new control strategies environmentally sound?

Vigne E.¹; Komar V.¹; Marmonier A.¹; Demangeat G.¹; Fuchs M.²; Lemaire O.¹

¹ INRA UMR 1131 Santé de la Vigne et Qualité du Vin, F-68000 Colmar, France

² Cornell University, 630 West North Street, Geneva, NY 14456, USA

Abstract

Grapevine fanleaf virus (GFLV) and *Arabidopsis mosaic virus* (ArMV) are responsible for fanleaf degeneration, which is the most severe virus disease of grapevines. These two viruses cause important economic losses by reducing grape yield, lowering fruit quality, and shortening the longevity of vines. They belong to the genus *Nepovirus* in the family *Comoviridae* and are exclusively transmitted by longidorid ectoparasitic nematodes.

We aim to develop new control strategies against fanleaf disease that are environmentally friendly. Genetic engineering is one of the strategies to develop virus-resistant grapevines, relying on the concept of pathogen-derived resistance through RNA interference and mediated by the transgenic rootstock. Cross-protection approach relies on the use of a mild viral isolate, in our case a mild isolate of GFLV or ArMV, to protect grapevines from a challenging infection by severe viral field isolates.

Regarding these new strategies, we are interested in determining if both strategies had any impact on the genetic variability of GFLV isolates and on the structure of GFLV populations. We also assessed if these strategies could assist the emergence of viable recombinants by the outcome of recombination events between RNAs from indigenous populations and viral transgene transcripts for GM rootstocks. Similar questions have been addressed for cross-protected grapevines to evaluate if recombination events could occur between RNAs from indigenous populations and RNAs from mild viral isolate.

Molecular variability studies, among GFLV isolates from 190 transgenic and 157 non transgenic plants in an open-field trial, indicate that transgenic grapevines did not assist the emergence of viable GFLV recombinants to detectable levels nor affecting the molecular diversity of indigenous GFLV populations during the trial period (1).

Recombination was assessed in a vineyard site in which grapevines cross-protected with mild strains GHu of *Grapevine fanleaf virus* (GFLV) or Ta of *Arabidopsis mosaic virus* (ArMV). SISCAN analysis confirmed a mosaic structure for two GFLV field isolates for which recombination sites were located in the movement protein and coat protein genes. One of the recombinants was found in eight grapevines that were in close spatial proximity within the vineyard site, suggesting its transmission by *X. index*. No interspecies recombination was detected between GFLV field isolates and ArMV-Ta. Altogether, our findings suggest that mild protective strains GFLV-GHu and ArMV-Ta did not assist the emergence of viable recombinants to detectable level during a 12-year cross-protection trial (2). A third study analyzing GFLV California field isolates showed interspecies recombination events between GFLV and *Arabidopsis mosaic virus* with crossover sites identified in RNA2 ORFs 2A^{HP} and 2B^{MP}; and intraspecies recombination events most frequently observed within gene 2C^{CP}. This study suggested that purifying selection and recombination are important evolutionary mechanisms in the genetic diversification of GFLV (3). The environmental impact of transgenic rootstocks on the nepoviral populations has however not yet been evaluated in-depth by a metagenomic approach. Consequently, an exhaustive study to address safety issues of virus-resistant or tolerant transgenic grapevine rootstocks, is required to identify potential environmental risks on the natural GFLV populations, in releasing such viral transgenes in the field.

Keywords: Grapevine, nepovirus, pathogen derived resistance, cross protection, virus population, GFLV, molecular diversity, recombination

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Deciphering the functional typology of resistance QTL through metabolomics Gravot A.¹; Wagner G.²; Lariagon C.³; Delourme R.³; Manzaneres-Dauleux M-J.²

¹ Université Rennes 1, UMR IGEPP, BP35327, 35653 Le Rheu, France

² AGROCAMPUS OUEST, UMR IGEPP, BP35327, 35653 Le Rheu, France

³ INRA, UMR IGEPP, 35327 Le Rheu, France

Abstract

The use of quantitative partial resistance, alone or in combination with specific resistance genes, seems to be an effective way to get varieties with an improved level of durability. With the assumption that the combination of QTL with different mechanisms or different effects on the pathogen can increase the sustainability of the resistance trait, the mechanisms underlying the involved QTL should be deciphered in order to rationalize their use in plant breeding. To date there is an insufficient understanding **of the cellular and molecular mechanisms associated to quantitative resistances, possibly due to the difficulty in cloning QTLs.**

Clubroot is caused by the telluric obligate biotrophe *Plasmodiophora brassicae* and is one of the most damaging diseases of cultivated Brassicas worldwide. The disease is characterized by the development of galls on the root system, seriously impairing nutrient uptake and resulting in important yield loss. The use of genetic resistances, particularly quantitative partial resistance, is considered as one of the most efficient control and sustainable strategies. We report here a combination of biochemical and quantitative genetics approaches to elucidate the complexity of cellular responses involved in the quantitative resistance in the clubroot/*Brassicaceae* pathosystem.

A “without *a priori*” strategy was developed, using targeted and untargeted metabolomics to identify metabolic biomarkers associated to the cellular response to clubroot infection, in several genotypes of *Brassica napus* and *Arabidopsis thaliana* harbouring various levels of susceptibility/partial resistance. This highlighted large biochemical similarities between susceptibility and resistance responses, and suggested the role of amino-acid, glutathione, glucosinolate, jasmonate and other still unidentified compounds in the susceptibility and resistance processes.

This metabolomic approach was also conducted in infected roots of segregating populations in *B. napus* and *A. thaliana*. Several colocalizations between QTL controlling symptoms and QTL controlling levels of metabolic biomarkers were identified. Resistance QTL were associated to different metabolic fingerprints, allowing i) to build a functional typology of several clubroot resistance QTL, and ii) to generate new hypotheses about the mechanisms underlying resistance/susceptibility to clubroot. Data from comparative genomics and metabolomics will be put in perspective to discuss the possible relationships between functional architecture of quantitative resistances in *Arabidopsis* and *B. napus*.

Keywords: Quantitative resistance, QTL, metabolomics, *Brassica*, *Arabidopsis*, *Plasmodiophora brassicae*, clubroot

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Long lasting rubber tree resistance to *Microcyclus ulei*

Garcia D.¹; Koop D.M.²; Pujade-Renaud V.³; Guyot J.; Mattos C.R.R.⁴; Seguin M.¹; Le Guen V.¹

¹CIRAD, UMR-AGAP/EGV, Avenue Agropolis, TA A-96/03, 34398 Montpellier Cedex 5, France

²Universidade Estadual de Santa Cruz (UESC), Departamento de Ciências Biológicas, Rod Ilhéus/Tabuna, Km 16, Salobrinho, CEP 45662-000 Ilhéus, BA, Brazil

³CIRAD, UMR-AGAP/EGV, 63000 Clermont-Ferrand, France

⁴CIRAD, UPR Bioagresseurs, BP 701, 97387 Kourou Cedex, French Guiana

⁵Plantações Michelin da Bahia, Cx Postal 2, CEP 45435-000 Ituberá, Bahia, Brazil

Abstract

The resistance of the MDF180 *Hevea brasiliensis* cultivar to South American leaf blight (SALB) caused by the fungus *Microcyclus ulei* has been studied under both controlled conditions of inoculation and natural infestation in the Brazilian state of Bahia. Inoculated isolates on this cultivar in most cases developed sporulating lesions and produced conidiospores, but at a moderate level, characteristic of a race non-specific, partial resistance. The teleomorph of *M. ulei* has never been reported on this cultivar, either in controlled conditions or in natural infestation, whereas it is commonly observed on other susceptible and partially resistant cultivars. This type of resistance has seldom been described for other pathosystems. MDF180 resistance now enduring for more than 30 years in areas very conducive to the disease, it can therefore be described as a durable resistance. To initiate a comprehensive approach of the resistance, five cDNA libraries were built and analyzed comparing up-regulated genes of MDF180 and PB314 (a susceptible genotype) from 6 h post-infection (hpi) to 58 days post-infection (dpi). We sequenced 8,027 expressed sequence tags (EST). After control of their quality, trimming and assembling, we obtained 1,165 singlets and 458 contigs. Based on sequence similarity 49% of the sequences could be assigned to functional categories, whereas 21% corresponded to already described sequences but with unknown function and 30% did not show any significant similarity with other sequences. Macroarray expression analysis allowed the identification of 311 genes differentially regulated during the infection process of both genotypes. Focusing on candidate genes associated with stress and defense, twelve genes were up-regulated in young lesions of MDF180 (e.g. NBS-LRR, cf5, Peroxydase, Pathogenesis-Related-Protein, cystatin) while seven genes appeared up-regulated in older lesions of PB314 (e.g. SOD, LRR, chitinase). These results provided a first contribution to the understanding of molecular mechanisms of the MDF180 resistance to South American leaf blight and provide information for the further exploration of signalization pathways involved in this durable resistance.

Keywords: South American leaf blight, *Hevea*, ESTs.

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Effect of androgenesis of wheat and triticale on *Stagonospora nodorum* blotch (SNB) resistance

Ziemichod M. E.; Arseniuk E.

Department of Plant Pathology, Plant Breeding and Acclimatization Institute – National Research Institute, Radzikow, 05-870 Blonie, Poland

Abstract

In modern plant breeding shortening of breeding cycle is a crucial factor. A double haploid technique offers such a possibility. Doubled haploid lines allow for phenotyping without recessive alleles and an influence of codominance. This is helpful in breeding aimed at a specific trait, e.g. resistance to pathogens.

The purpose of the study was to compare resistance of dihaploid lines and conventional donor lines/cultivars of winter and spring types of wheat and triticale to *Stagonospora nodorum* blotch.

Production of dihaploid lines of wheat and triticale:

To produce DH lines of wheat and triticale F1 kernels were planted to soil pots and grown under controlled environment conditions. Spikes were collected in mid-uninucleate stage of microspores, and preserved for 7 to 10 days in a fridge. Cold treated spikes were subsequently sterilized with sodium hypochloride. Anthers were removed under sterile conditions and plated on CLM (wheat) or A (triticale) media. Subsequently plates with anthers were incubated at 26 °C. After about 6 weeks emerged calli were transferred onto R1 medium. Callus derived plants were further transferred to R4 rooting medium. The regenerated plants were then vernalized, transferred to a soil in pots and grown until grain production.

Field experiments at adult plant growth stages:

Produced seed of DH lines and conventional wheat and triticale cultivars of winter and spring types was used to conduct a field test for resistance to *Stagonospora nodorum* blotch (SNB). Random 1m² triplicate plots for each DH line/variety were planted. Plants were inoculated three times with a water suspension of *S. nodorum* pycnidial spores (3 mio spores/ml) at GS 45, 59, 65 (Zadoks scale). Before inoculation control plots were sprayed with Tilt 250 EC (0,1 % propiconazole). Disease (*Stagonospora nodorum* blotch) rating on 9 digit scale, respectively, (1 – susceptible, 9 – resistant) on leaves and heads was done at weekly intervals from the appearance of first disease symptoms to the plant death. Also plant height (cm) and heading dates in Julian days were measured.

In conclusion, it could be stated that DH lines on average appeared to be more resistant to SNB, than the recombinant ones.

Keywords: Doubled haploid, *Stagonospora nodorum*

Induced resistance in sunflower: defense mechanisms against a biotrophic and a necrotrophic plant pathogen

Körösi K.; Bán R.; Baglyas G.; Virányi F.

Plant Protection Institute, St. István University, Gödöllő, Páter K. u. 1., Gödöllő, Hungary, Körösi.Katalin@mkk.szie.hu

Abstract

Downy mildew (*Plasmopara halstedii* (Farl.) Berl. et de Toni) and white rot (*Sclerotinia sclerotiorum* (Lib.) de Bary) are significant diseases of sunflower. *S. sclerotiorum* causing devastating soft rot has a wide host range as well as able to survive for many years as hard sclerotia which makes control of the disease particularly difficult. Although *P. halstedii* can be effectively controlled by using resistant cultivars and seed dressing with fungicides, protection may be hindered by the high genetic variability of the fungus. Since control measures against these pathogens are not satisfactory (white rot) or limited in time (downy mildew), alternative and supplementary methods such as the use of chemical or biological resistance inducers are required. Goals of the present study were to examine the effectiveness of abiotic (chemical) and biotic (mycorrhizal fungi) resistance inducers on the restriction of disease, and to compare the mechanisms of induced resistance in case of a biotrophic (*P. halstedii*) and a necrotrophic (*S. sclerotiorum*) pathogen.

Earlier studies showed that BTH (benzothiadiazole) significantly reduced the appearance of downy mildew symptoms of pretreated and infected plants and enhanced some defense-related enzyme activities in susceptible sunflowers. In this work we tested the effect of BTH and mycorrhizal fungi on downy mildew and white rot diseases in susceptible plants under greenhouse conditions. We wanted to know, whether BTH and/or mycorrhizal fungi are able to restrict disease development in plants, and if so, are there differences in the mode of action between these two pathosystems. To answer these questions, pathogenic spread in plants (macroscopic and microscopic observations) and changes in the activities of two significant antioxidant enzymes (polyphenol oxidase, PPO and guaiacol-dependent peroxidase, POX) were examined following pre-treatment by inducers and inoculation.

Histological observations were undertaken by fluorescence microscopy. Samples of sunflower seedlings were taken at intervals and a series of cross-sections were cut from the hypocotyl. Sections were then observed under microscope for detecting fungal structures and host cell responses. Enzyme activity assays were carried out by using spectrophotometric methods. POX and PPO activity were determined as described by Rathmell and Sequeira (1974) and using a modified procedure of Fehrmann and Dimond (1967), respectively.

Fluorescence microscopical observations revealed a significant inhibition of colonization by either pathogen following a treatment of BTH and/or mycorrhiza in susceptible sunflowers. In addition, defense-like host responses such as cell necrosis (downy mildew) and fluorescent cell clusters (white rot) could be detected in these plant tissues. BTH treatment increased PPO and POX activities in both inoculated (either downy mildew or white rot) and non-inoculated plants. Enzyme activities were higher in the BTH and mycorrhizal treated, inoculated plants as compared to non-inoculated ones in both plant-pathogen interactions.

Investigations on the chemical and biological resistance inducers so far are promising and might suggest the potential of their future use as additional control agents against downy mildew and white rot in sunflower production.

Research was supported by the TÁMOP 4.2.2/B-10/1-2010-011 „Development of a complex educational assistance/support system for talented students and prospective researchers at the Szent István University” project and OTKA (81209K).

Keywords: *Plasmopara halstedii*, *Sclerotinia sclerotiorum*, BTH, mycorrhizal fungi, antioxidant enzymes

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Arabidopsis Mitogen Activated Protein Kinase 4 and its homologs positively regulate defense responses against *Botrytis cinerea* in different plant species

Nasar V.¹; Fengming S.²

¹Atta ur Rahman School of Applied Biosciences, National University of Sciences and Technology, Islamabad, Pakistan.

²National Key Laboratory of Rice Biology, Institute of Biotechnology, Zhejiang University, Hangzhou, P.R.China.

Abstract

Mitogen-activated protein kinases (MPKs) play important roles in biotic and abiotic stress responses. In the present study, we identified the tomato SIMPK4, a possible homolog of Arabidopsis AtMPK4, and functionally characterized the tomato SIMPK4 and Arabidopsis AtMPK4 for their possible roles against *Botrytis cinerea*, a necrotrophic fungal pathogen causing grey mold disease. Expression of SIMPK4 and AtMPK4 was induced by infection with *Botrytis cinerea* and expression of SIMPK4 was also induced by jasmonic acid. Knockdown of the endogenous SIMPK4 expression via virus-induced gene silencing in tomato plants (TRV-SIMPK4) resulted in increased susceptibility to *Botrytis* infection. Similarly, knockdown of the endogenous NbMPK4 in *Nicotiana benthamiana* using TRV-SIMPK4 construct also resulted in increased susceptibility to *B. cinerea*. Expression of defense-related genes *SIPR1a* and *SIPR1b* were attenuated in SIMPK4-silenced plants. We generated dexamethasone-induced AtMPK4 knockdown transgenic lines and found that knockdown of the endogenous AtMPK4 gene expression led to enhanced susceptibility to *B. cinerea* as compared to vector-transformed wild type control. Taken together, our results suggest an evolutionary conserved role for MPK4 gene across different plant species in defense response against *B. cinerea*.

Keywords: *Arabidopsis* MPK4, Tomato MPK4, MAPKs in plant defense

Expression of pathogen *Fusarium moniliforme* and its biochemical response in early stages of maize (*Zea mays* L.)

Singh N.

Directorate of Maize Research, Pusa Campus, New Delhi, INDIA- 110012

Abstract

Stalk rot caused by *Fusarium moniliforme* Sheldon is a destructive disease in maize incurring a yield loss of 20-30 percent. *Fusarium moniliforme* can be either an endophyte of maize, causing no visible disease, or a pathogen-causing disease of stalks, roots and seedlings. In experiment, seeds were soaked in three replications in spore suspension overnight and incubated at 28°C and 70% Relative humidity for seven days. Observation on extent of germination, mycelia growth and seed rotting were recorded. In seedling experiment, surface sterilized seeds were sown in six replications with five seedlings per pot and allowed to grow for three weeks in glass house conditions. Twenty one days old seedlings were sprayed with *F. moniliforme* spore suspension @ 2ml/seedling and studied for disease reaction. The biochemical assay of total phenol and soluble sugar was also done to further confirm the result obtained from pathogenicity test. In seeds experiment, though seeds were surface sterilized there was an attack

of pathogen in varied degree ranging from high surface infection leading to seed rotting (36.6%) to low surface infection (16.7%) and in some case no infection (46.7%). The seed rotting may be due to presence of pathogen in kernel by infection *via* silk channel or internal and systemic infection of the corn plant. But the possibility of mechanical injury due to shelling seeds paving entry to pathogen spores in the germinating seeds may not be ignored. In seedling experiment, along with significant symptoms *viz.*, tip drying, necrotic lesions, chlorotic bands and yellowing of margins, the appearance of asymptomatic seedlings was the major finding. As *Fusarium moniliforme* spores are widespread and move easily by air resulting in increased seedling symptoms, inoculation technique by spraying is effective in developing symptoms and screening for *F. moniliforme* infection at seedling stage. The expression obtained was confirmed by biochemical investigation wherein the contents of total soluble sugars and phenol were estimated in seedling samples. The asymptomatic showed the symptomless colonisation of fungus as indicated by the higher contents of phenol (5.24mg/g) and sugar (4.28mg/g) as compared to control (2.18, 2.84mg/g, respectively). This was further confirmed by microscopic studies of the asymptomatic seedling, showing the colonisation of fungi without any external symptoms. The appearance and non-appearance of symptoms in infected host can mislead the identification of resistant genotypes. This study led down a pathway to select crop varieties more carefully as results embarked no assurance of complete disease-free plants. Symptomless plants remained symptomless throughout the observation period; this indicates that the symptomless state persists beyond the seedling stage and could contribute, without visual signs, to the total mycotoxin contaminants of maize both before and during kernel development. The development of appropriate control and detection measures for virulence is required. Hence, the inoculation techniques and stage of screening (seed to mature kernel) should be in line with the life cycle of pathogen and changing climate.

Keywords: Maize, *Fusarium moniliforme*, Total soluble sugar, Total phenol

In Search of Anthracnose Susceptibility Host Factors in Common bean

Fraire-Velázquez S.; Ramírez-Cabral N.; De La Cruz-Rodríguez Y.; Vázquez-Herrera C.

Abstract

The fate of a compatible plant–pathogen interaction is often determined by virulence pathogen factors and susceptibility host factors. Susceptibility host factors means that the host exhibits determinants required for growth and reproduction of pathogen and plant tissue colonization that conduits to plant disease, all in a sense of plant cooperative interaction. A subset of plant pathogenic fungi produce a wide range of compounds that act as pathogenicity determinants referred as host-selective toxins (HST) and HSTs are usually active against plants susceptible to pathogen that synthesizes them [1]. The mutation, downregulation or the silencing of these susceptibility genes and the corresponding molecular mechanisms could bring new ways of disease resistance. Common bean is an important protein source in the entire world and this crop is seriously affected by the anthracnose disease caused by *Colletotrichum lindemuthianum*. The object of this work is to search and identify host up-regulated genes in a susceptible interaction of a *Phaseolus vulgaris/C. lindemuthianum* pathosystem, and subsequently, the silencing or over-expression of these genes to analyze the pathosystem behavior, in order to decipher some specific plant molecular mechanisms that characterize the molecular nature of a compatible interaction. By subtractive hybridization, a collection of 196 clones of cDNA fragments were obtained [2], and by reverse Southern in two-ways (in first instance with total RNA from plant in incompatible interaction as probe, and then with RNA from plant in compatible interaction), 48 cDNA fragments show over-expression in compatible interaction: The sequenced fragments gave 38 contigs and from analysis in NCBI data Bank and Gene Ontology algorithms, eight functional groups were obtained, 39% of unknown function, 16% involved in metabolism, 16% in signal transduction, 9% in regulation of transcription. By bioinformatic analysis in eight clones, four sequences exhibit homologies with hypothetical proteins from several plants including *Glycine max* and *Arabidopsis thaliana*. One sequence corresponds to rubisco activase (Rca1) from *P. vulgaris*; in the others, one corresponds to an

E3 ubiquitin-protein ligase (SINAT5-like), and the last one is a squamosa promoter-binding protein 1-like, these two last both from *Glycine max*. In GO (AmiGO browser), four clones with biological function or biological process not determined; in the others, rubisco activase with the known biological process, furthermore involved in defense response to bacterium, in response to jasmonic acid stimulus, and noteworthy, in negative regulation of defense response; other clone involved in ubiquitin-dependent protein catabolic process, and the last one adscribed to regulation of transcription. In summary, that means new candidates of host sensitivity gene products in common bean to *C. lindemuthianum*. Experiments of silencing by RNAi of these genes focused on transcription factors and signaling transduction, and their effects in plant in the same pathosystem are underway.

Keywords: anthracnose susceptibility, susceptibility determinants, compatibility determinants, susceptibility genes.

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Tracing genes for resistance to apple scab disease *Venturia Inaequalis*

Madduri M.

Abstract

Apple scab (*Venturia inaequalis*) is one of the major apple diseases. Growers are spraying up to 20 times per year to control the disease, hence the development scab resistant cultivars is crucial to reduce the fungicidal usage. Many resistant genes have already been identified, some of which still have a wide spectrum of resistance to current fungal races. Based on current knowledge, cultivars with stable resistances can be produced by pyramiding several resistance genes into one cultivar. Therefore the focus of this project is to explore sources of scab resistance that have been effective for over 30 years in the Wageningen University and Research Centre (WUR) breeding program. We aim to dissect the genetic base of these resistances and find linked molecular markers to pyramid them with already available genes through marker-assisted selection. Here we report on a new gene segregating in a family derived from *Malus micromalus*, but originated from the susceptible grandparent 'Cox's Orange Pippin'.

Keywords: Apple scab, Disease resistance, Pyramiding, MAS

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Sunflower Downy Mildew: Microscopic and imagery studies of *Plasmopara halstedii* infection, inventory of pathogenicity effectors and map-based cloning of a quantitative disease resistance locus

Pichon M.¹; Gascuel Q.¹; Martinez Y.²; Munos S.¹; Pouilly N.¹; Blanchet N.¹; Boniface M. C.¹; Godiard L.¹; Vincourt P.¹

¹Laboratoire des Interactions Plantes-Microorganismes (LIPM), UMR441, INRA-CNRS, F-31326 Castanet-Tolosan, France

²CNRS, FR3450, BP42617, F-31326 Castanet-Tolosan CEDEX, France

Abstract

Downy mildew in sunflower (*Helianthus annuus*) is caused by the biotroph oomycete *Plasmopara halstedii*. The resistance to this disease is controlled by major dominant genes denoted PI and QTLs conferring quantitative resistance. A quantitative resistance to the 710 race of *P.halstedii* is driven by 3 QTLs on LG7, 8 and 10 (Vincourt et al., TAG 2012). The major QTL, QRM1 of LG10 explains 65% of the quantitative resistance in a growth chamber assay. In order to understand the mechanisms conferred by QRM1, a map-based cloning approach of the QTL is underway via the development of Near Isogenic Lines for QRM1.

Massive cDNA sequencing from infected plants and isolated germinating spores of *P. halstedii* allowed the identification of many putative RXLR and CRN pathogenicity effectors (As-sadi et al., BMC Genomics 2011). In planta temporal expression studies of these effectors are underway. In the same time, over-expression of 5 CRNs in sunflower leaves, using a leaf infiltration method developed in our laboratory with LBA4404 *A. tumefaciens* strain has been performed. Elsewhere, the genome sequencing of 7 races of *P. halstedii* should help us in describing the variability of *P.halstedii* CRN and RXLR effectors.

Whereas the symptoms and the genes controlling resistance or susceptibility are well described in the literature, the histological aspects of the infection process of sunflower by *P.halstedii* remain fragmentary. By using Scanning Electron Microscopy (SEM), light microscopy, and confocal imagery we followed pathogen development in the early stages of the interaction, in resistant and susceptible lines.

Keywords: Sunflower, *Plasmopara halstedii*, effectors, QTLs, microscopy

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BioMolChem, a tool to assess the defence status of grapevines after stimulation, or not, of cultivar or resistant genotypes: from genes to the field

Corio-Costet M.F.¹; Lambert C.²; Cluzet S.²; Merdinoglu D.³; Dufour M.C.¹

¹INRA, UMR Santé et Agroécologie du vignoble (SAVE-1065), ISVV, BP 81, 71 rue Bourlaux, 33883 Villenave d_'Ornon, France. coriocos@bordeaux.inra.fr.

²Université de Bordeaux, ISVV, Gesvab-EA 3675, CS 50008, 33882 Villenave d_'Ornon, France,

³INRA, UMR1131 Santé de la Vigne et Qualité du Vin INRA, BP 20507, 68021 Colmar, France

Abstract

Stimulating plant defences, or resistant plant varieties is of interest as an alternative method to limit pesticide use in agriculture. To assess the defence status of the grapevine we have developed a triple approach called BioMolChem:

- **Biological** tests that measure the efficacy of the grapevine defenses against two major biotrophic pathogens (*Erysiphe necator*, *Plasmopara viticola*),
- **Molecular** assays by q-RT-PCR show the expression patterns (over-expression or repression) of twenty genes involved in the grapevine defences, and these can then be correlated, or not, with the protection level,
- **BioChemical** analyses by HPLC of phenylpropanoides quantify and to identify molecules of interest, and correlate them with specific gene expression (stilbene biosynthesis) and with the acquired protection.

This tool was tested on leaves (cv. Cabernet-Sauvignon) after stimulation by different elicitors (benzothiadiazole, phosphonates), on grapevine genotypes resistant to powdery and downy mildew, and in the vineyard. We obtained correlations between the expression of PR-protein genes and genes coding for biosynthesis pathways or (stilbene, tryptophan) or with the efficacy of protection. Similarly, there were correlations between the presence of known and unknown molecules, and the efficacy of protection. We found that resveratrol, a well known phytoalexin of the grapevine, is a good marker of defence status, but is not a good marker of protection. Today, we therefore have a tool for understanding the defence and protection status of the grapevine in laboratory or in field experiments.

Keywords: plant defence, defence status, elicitor, resistant genotypes

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Dufour *et al.*, (2012), Plant Pathol. Doi :10.1111/j.1365-3059.2012.02628x

Ultrastructural studies the participation of organelle in the TRV infection of tobacco and potato

Garbaczewska G.¹; Chouda M.¹; Otulak K.¹

¹Warsaw University of Life Science – SGGW, Faculty of Agriculture and Biology, Department of Botany, Nowoursynowska Str.159, 02-776 Warsaw, Poland; grazyna_garbaczewska@sggw.pl

Abstract

The objective of our ultrastructural studies was to participation of cellular organelle during the process infection of *Nicotiana tabacum* cv. *Samsun* and *Solanum tuberosum* cv. *Glada* (susceptible) and cv. *Sokół* (resistant) with *tobacco rattle virus*.

Tobravirus are characterized as having a genome consisting of two species of (+) ssRNA, RNA 1 and RNA 2, contained in straight rod-shape particles of two lengths. The long (L) ones, infective, 180 to 197 nm long, encode proteins involved in multiplication of virus genome and intercellular transport (Mac

Farlane 1999). The short (S) ones are non-infective, 43-114 nm long, encode capsid proteins (CP) of both particles and two nonstructural proteins involved in transmission by nematodes (Hernandez et al. 1997). Plants with four levels of leaves were inoculated mechanically with PSG strain of TRV. Two weeks after infection tobacco plants cv. *Samsun* and potato cv. *Glada* responded with spot surface necroses on inoculated leaf blades. Four weeks after infection the systemic response included not only leaves but also surface necroses of stems and underground organs of potato. Inoculated leaf blades and petioles of potato cv. *Sokół* two weeks after infection were necrotized. Our findings suggested systemical transfer by phloem and xylem of TRV PSG in susceptible hosts as a result of nematode vector transmission. (Garbaczewska et al. 2012).

Ultrastructural analysis presented that TRV particles of two lengths are organized in encapsidated and noncapsidated forms in dispersed and regular inclusion types. Recent investigations have revealed presence of TRV particles only in cytoplasm of mesophyll cells and replication areas are connected with cytoplasm. We observed intense, significant changes in internal structure of chloroplasts, mitochondria and cell nucleus. It was noticed considerable deformations of mitochondria connected with its enlargement and the presence of internal vesicles. For the first time it was observed TRV particles in mitochondria, chloroplasts and cell nucleus. The disorganized chloroplasts lost a typical lamellas arrangement whereas TRV particles of two lengths are visible in stroma. Inside cell nucleus was observed electron-transparent areas with fibril material and TRV particles.

Keywords: *tobacco rattle virus*, ultrastructure, *nucleus*, mitochondria, chloroplasts

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The ultrastructural events in hypersensitive and compatible reactions of potato and tobacco infected with PVY necrotic strains

Otulak K.¹; Garbaczewska G.¹

¹Warsaw University of Life Science – SGGW, Faculty of Agriculture and Biology, Department of Botany, Nowoursynowska Str.159, 02-776 Warsaw, Poland; katarzyna_otulak@sggw.pl

Abstract

Potato plants cv. *Rywal* with hypersensitivity gene *Ny-1* (Szajko et al., 2008) infected with PVY^N or PVY^{NTN} reacted in local necroses 3 days after infection. Potato virus Y (PVY) particles were found in epidermis, mesophyll, phloem and xylem tracheary elements during HR and compatible response. Noncapsidated virus particles forms were observed already 10 hours after infection by using electron microscopy in situ. Capsid protein on one terminus of noncapsidated virus particles was located 5 days after inoculation with the use of immunogold labeling method. Whereas cytoplasmic inclusions were observed for the first time 24 days after infection during hypersensitive response (Otulak & Garbaczewska, 2010). In compatible tobacco cv. *Samsun* and potato cv. *Igor* tissues response to PVY inoculation systemic necrosis were presented 15 days after infection. Ultrastructural studies showed that ER may take part in PVY replication and also inclusions formation. Observed cytopathological changes and virus particles indicate that cell nucleus and mitochondrion might participate in PVY life

cycle. In HR as well as in compatible response PVY particles were found in plasmodesmata and also in vascular tissues. Virus particles and PVY cytoplasmic inclusions observed in phloem and xylem gives ground to conclude that even in case of resistant potato variety PVY is able to systemic transport through phloem and xylem.

Keywords: virus Y, ultrastructure, hypersensitive response, immunolabeling

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Genome-wide transcriptome analysis of the *Arabidopsis thaliana*_Phytophthora parasitica interaction: towards the characterization of genes involved in plant susceptibility to soilborne oomycetes.

Le-Berre J-Y.; Gourgues M.; Morabito C.; Keller H.; Panabieres F.; Attard A.

J-Y Le-Berre, M. Gourgues, C. Morabito, H. Keller, F. Panabieres, and A. Attard

UMR-Institut Sophia Agrobiotech, INRA1355-CNRS7254-Université Nice–Sophia Antipolis, BP 167, 400 Route des Chappes. 06903 Sophia Antipolis Cedex, France. E-mail: agnes.attard@sophia.inra.fr

Abstract

Oomycetes from the genus *Phytophthora* are fungus-like pathogens that are devastating for agriculture and natural ecosystems. Due to their particular physiological characteristics, no sustainable management strategy of these microorganisms is currently available. Deciphering molecular mechanisms that govern interactions between *Phytophthora* spp. and their host plants may thus provide clues to develop new, efficient plant protection approaches. In this context, our laboratory aims at understanding the molecular events underlying the onset of the compatible interaction between plant roots and *Phytophthora* species. For this purpose, we took advantage of a system involving the model plant *Arabidopsis thaliana* and the wide host range pathogen, *Phytophthora parasitica*. We monitored *A. thaliana* transcriptome modulations from root penetration by *P. parasitica* motile zoospores to the switch from biotrophy to necrotrophy. We showed that the interaction transcriptome is highly dynamic, and that many biological processes are regulated as soon as the first contact between plant roots and the oomycete occurs. Among them, responses involving the hormones ethylene (biosynthesis and response) and auxin (cellular transport) are highly modulated. To identify plant functions that are manipulated by *P. parasitica*, we selected thirteen *A. thaliana* candidate genes that show a significant transcriptional up-regulation during the first hours of infection. The characterization of corresponding knock-out mutants revealed two genes contributing to susceptibility, and three to resistance of *A. thaliana*. These results will be presented and their potential use for plant protection purposes will be discussed.

Keywords: Root, Susceptibility, Oomycete, *Phytophthora*, Penetration

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An *Arabidopsis thaliana* glycosyltransferase contributes to downy mildew disease.

Allasia V.; Ponchet M.; Panabières F.; Keller H.

UMR-Institut Sophia Agrobiotech, INRA1355-CNRS7254-Université Nice-Sophia Antipolis, Sophia Antipolis, France.

Abstract

UDP-glycosyltransferases (UGTs) are enzymes present in all living organisms. They catalyze the transfer of a monosaccharide from an activated donor to an acceptor, thereby forming a glycosidic link. Hormones, secondary metabolites involved in responses to biotic and abiotic stress, and xenobiotics such as herbicides are substrates for UGTs in plants. Glycosylation may activate or inactivate these compounds, but also increase their hydrophilic properties and thus regulate the subcellular compartmentalization. During interactions between plants and biotrophic pathogens, UGTs ensure the cellular homeostasis between active (cytoplasmic) and inactive (vacuolar) forms of the defense hormone salicylic acid (SA). We identified an *Arabidopsis thaliana* UGT, which is transcriptionally activated during the interaction with the obligate biotrophic oomycete *Hyaloperonospora arabidopsidis* (Hpa). A knock-out mutation of the UGT reduces plant susceptibility to the downy mildew pathogen, whereas its overexpression stimulates the development of disease. In the absence of infection, mutant plants present normal phenotypes and are not altered in immune responses, whereas *Hpa* infection leads to a substantial accumulation of SA and a rapid stimulation of SA-dependent defenses. Mutants are thus “primed” for defense. Our analyses, including a comparison of the global transcriptome between wild-type and mutant plants, indicate that the UGT negatively regulates the defense status of plant cells. An inactivation of orthologs in agronomically important plants might represent an alternative to “prime” plants for defense, thus avoiding the fitness costs that concur with activated immune responses.

Keywords: Salicylic acid, defense, priming, susceptibility, oomycete.

Four secreted proteins transiently accumulated during the penetration process of *Phytophthora parasitica* facilitate plant infection.

Evangelisti E.; Minet-Kebdani N.; Attard A.; Kuhn M-L.; Panabières F.; Gourgues M.*

UMR-Institut Sophia Agrobiotech, INRA1355-CNRS7254-Université Nice-Sophia Antipolis, BP 167, 400 Route des Chappes. 06903 Sophia Antipolis Cedex

*corresponding author: mathieu.gourgues@sophia.inra.fr

Abstract

Oomycetes from the genus *Phytophthora* are filamentous pathogens that cause major damages for agriculture and natural ecosystems. The development of efficient strategies against these micro organisms implies to dissect the molecular mechanisms that determine the interaction between *Phytophthora* spp and their host plants. The early steps of infection, especially root penetration and colonization are poorly documented. To gain insights on these crucial events, we analyzed the appressorium mediated penetration of *Arabidopsis thaliana* roots by the soilborne pathogen *Phytophthora parasitica* through a transcriptome analysis. Gene expression was shown to be highly regulated during the first hours of the interaction. Notably, 124 *P. parasitica* transcripts transiently

accumulated during penetration were identified. We selected four candidate genes belonging to two families of secreted proteins for a subsequent functional analysis. The first candidate (PpSCPL1) contains a SCP-like extracellular domain. Such proteins exist in all living organisms. They were shown to control various processes from cellular adhesion to cell differentiation but the molecular function of the SCP domain is still unknown. Accumulation of the PpSCPL1 protein in tobacco leads to an enhanced colonization by Tobacco Rattle Virus and abolishes plant cell death caused by different elicitor proteins suggesting the ability of the protein to contribute to plant susceptibility. The second set of candidate genes belongs to the RXLR effector family. These proteins are secreted by oomycetes and imported into the plant cell where they modulate defense responses. The three Penetration Specific Effectors (PSE1, PSE2 and PSE3) analyzed abolish plant cell death caused by elicitors in tobacco. Moreover, constitutive expression of PSE1 and PSE2 leads to increased susceptibility of *Arabidopsis thaliana* to *Phytophthora parasitica*. The molecular function of PSE2 is still unknown while PSE1 is able to modulate auxin hormone content to enhance plant susceptibility. Strong developmental perturbations following expression of PSE3 in *A. thaliana* also suggests a perturbation of hormone signaling pathways. This study shows that RXLR effectors expressed during the first hours of the interaction modulated general features of plant development to favor infection.

The identification of plant targets of these effectors in cultivated plants such as tobacco and tomato, and the search for variants that could not be affected by the pathogen proteins in germplasm will lead to the development of new strategies to control oomycete diseases.

Keywords: *Phytophthora*; appressorium; *Arabidopsis*; tobacco; effectors; pathogenicity; hormones

Identification of effectors secreted by the fungal pathogen *Fusarium graminearum*, main causal agent of FHB on wheat

Lecomte Ph.^{1,2}; El Hajj Assaf Ch.^{1,2}; Da Rocha M.³; Chetouhi Ch.^{1,2}; Danchin E.³; Langin T.^{1,2}

¹INRA, UMR1095 GDEC, F-63039 Clermont-Ferrand, France

²Université Blaise Pascal, UMR GDEC, F-63039 Clermont-Ferrand, France

³UMR 1355 INRA-CNRS-Université de Nice Sophia-Antipolis, Institut Sophia Agrobiotech, F-06903 Sophia-Antipolis, France

Abstract

Fusarium head blight (FHB), caused by several *Fusarium* spp., is a widespread and destructive disease of wheat (*Triticum aestivum* L.), barley, and other small-grain cereals. These pathogens infect spikes and reduce grain yield and quality. Moreover, *Fusarium* spp. that cause FHB, produce trichothecene mycotoxins such as deoxynivalenol (DON) and nivalenol (NIV), which are toxic to humans and other animals. To control this dangerous disease, several strategies could be employed, such as application of fungicides, use of biological antagonists and breeding of genetically resistant cultivars. In order to develop new strategies able to insure wheat resistance sustainability, it appears to be important to understand at the molecular level the interaction between the wheat and *Fusarium graminearum*, the main causal agent of FHB on wheat. Successful pathogens have evolved strategies to manipulate their host and promote disease. Effectors are low molecular weight proteins secreted during infection to interfere with host immune systems and host metabolism in order to promote disease. To define the repertoire of fungal effectors produced by *F. graminearum* during infection, we have developed an *in vitro* pathosystem. 236 fungal secreted proteins have been identified by MS/MS. *In silico* analysis showed that 60% of these proteins have a predicted secretion peptide signal. A gene ontology analysis led to cluster these proteins in 8 major classes: degradation of carbohydrates and proteins, including enzymes targeting plant cell wall (60%), fungal defense and oxidative stress detoxification (22%); basal metabolism (7%); and unknown proteins (5%). Among these fungal proteins, 14 proteins were selected

as putative effectors based on their (a) their lack of similarity to known proteins, (b) their similarity to effectors already identified in other fungal pathogens, (c) the presence of interesting protein motifs (NLS, ...), and (d) their molecular weight. These candidates will be further characterized (targeted gene knock-out experiments to test their virulence or avirulence functions, *in planta* localization, identification of potential plant targets).

Keywords: FHB disease, *Fusarium graminearum*, fungal effectors, plant susceptibility.

Oomycete and fungal pathogens recruit a plant receptor to establish disease in *Arabidopsis thaliana*

Hok S.¹; Allasia V.¹; Naessens E.¹; Ribes E.¹; Andrio E.¹; Danchin E.¹; Panabières F.¹; Attard A.¹; Voll L.¹; Saur A.²; Hückelhoven R.³; Grill E.⁴; Marco Y.⁵; Barlet X.⁵; Clément M.⁵; Keller H.¹

¹UMR-Institut Sophia Agrobiotech, INRA1355-CNRS7254-Université Nice-Sophia Antipolis, Sophia Antipolis, France;

²Friedrich-Alexander-Universität Erlangen-Nuremberg, Lehrstuhl für Biochemie, Erlangen, Germany;

³Technische Universität München, Lehrstuhl für Phytopathologie, Freising-Weihenstephan, Germany;

⁴Technische Universität München, Lehrstuhl für Botanik, Freising-Weihenstephan, Germany;

⁵Laboratoire des Interactions Plantes Microorganismes, UMR CNRS-INRA 2594/441, Castanet-Tolosan, France;

⁶UMR 6191 CEA, Centre National de la Recherche Scientifique, Laboratoire de Biologie du Développement des Plantes, Université d'_Aix-Marseille, Saint-Paul-lez-Durance, France.

Abstract

Plant leucine-rich repeat receptor kinases (LRR-RK) are a family of membrane proteins that coordinate developmental processes and innate immune responses. We previously identified the *Arabidopsis* LRR-RK Impaired Oomycete Susceptibility 1 (IOS1), which is recruited by oomycete pathogens to promote disease. Here, we show that a *ios1* mutant is less susceptible to infections by different filamentous biotrophic pathogens, and that it is hypersensitive to the phytohormone, abscisic acid (ABA). We demonstrate that IOS1 negatively regulates the ABA signaling pathways in plants, and that oomycete and fungal pathogens recruit the receptor to downregulate ABA signaling upon infection. IOS1 is encoded by a gene that successively duplicated to give rise to a cluster of 11 genes, indicating strong selective pressure on members of the *IOS1* family. IOS1 is atypical by possessing an extracellular domain that is composed of LRR motifs and a large malectin-like (ML) domain. While LRRs are involved in protein-protein interactions, ML-domains allow to bind carbohydrates. We provide evidence that the IOS1 exodomain is essential for receptor function during plant-microbe interactions, and we propose to exploit IOS1 characteristics in a gene therapy for increasing plant disease resistance.

Keywords: Susceptibility, hormone, signaling, competitiveness, oomycete

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Correlation between coat protein aggregation and resistance of tomato to *Tomato yellow leaf curl virus* (TYLCV)

Gorovits R.¹; Kolot M.²; Moshe A.¹; Sobol I.¹; Czosnek H.¹

¹Institute of Plant Sciences and Genetics in Agriculture and the Otto Warburg Minerva Center for Agricultural Biotechnology, The Robert H. Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100, Israel

²Department of Biochemistry, Tel-Aviv University, Ramat Aviv 69978, Israel

Abstract

Sedimentation of leaf extracts of tomato plants infected with *Tomato yellow leaf curl virus* (TYLCV) through sucrose gradients showed that the virus coat protein (CP) forms aggregates of increasing size with the progress of infection. Development of small, mid-sized and large CP aggregates has been confirmed by *in situ* immuno-staining of leaf and sap samples. At early stages of infection, the signals appeared as discrete punctuate spots in the cytoplasm, while at the later stages aggregates of increasing sizes localized in cytoplasm and nuclei. Infectious whitefly-transmissible particles were found only in large nuclear insoluble aggregates. We tested the hypothesis that the extent of aggregation reflects the degree of resistance of the host plant to the virus. CP patterns were compared in two inbred lines derived from the same breeding program, one susceptible (S), the other resistant (R) to TYLCV. While the size of CP aggregates increased with time upon whitefly-mediated infection, velocity of aggregation was lower in R than in S plants. The degree of CP aggregation was modulated by changing experimentally the level of resistance/susceptibility of the R and S genotypes: intermediate midsized CP aggregates correlated with resistance, large insoluble aggregates with susceptibility. CP expressed in *E. coli* could be degraded by proteases found associated with midsized plant CP aggregates. Complexes between the viral CP and the plant protease FtsH were identified *in vitro*. We propose that sequestering viral CP into midsized aggregates and retarding the formation of large complexes containing infectious particles is part of the response of R plants to TYLCV infection.

Keywords: virus resistance aggregation

The Agc2 Kinase Oxi1 regulates salicylic acid-dependent plant immunity and cell death programs in *Arabidopsis*

Garcia A.V.¹; Forzani C.²; Yoshimoto K.³; Balagué C.⁴; Bueso E.¹; Colcombet J.¹; Hirt H.¹

¹URGV - Plant Genomics Research, France

²Max Perutz Laboratories, Austria

³IJPB – INRA Versailles, France

⁴LIPM - Laboratory of Plant Microbe Interactions, France

Abstract

In animals, the kinases PDK1 and TOR regulate AGC kinases in response to growth factors and environmental stress, to modulate cell size, cell number and cell death and thereby influence growth. *Arabidopsis* OXI1 is a Serine/Threonine kinase that belongs to the AGC family and is rapidly activated by oxidative stress and various signals mimicking pathogen attack (Garcia *et al.* 2012, Rentel *et al.* 2004). OXI1 is required for complete resistance against biotrophic pathogens and for the growth promotion conferred by the basidiomycete *Piriformospora indica* (Camehl *et al.* 2011, Garcia *et al.* 2012). Furthermore, *oxi1* mutant plants display reduced activation of the stress-induced MAP kinases MPK3 and MPK6 (Rentel *et al.* 2004). Whereas animal AGC kinases are central regulators of cell death programs and growth in response to environmental stresses and growth factors, little is known about the function of AGC kinases in these processes in plants. To further assess the function of Arabidopsis

OXI1, we generated transgenic lines expressing tagged *OXI1* under its own promoter and selected lines with different levels of protein accumulation. Our results show that *OXI1* over expression leads to developmental defects, including reduced growth and severe leaf necrosis. The appearance of necrotic lesions correlates with the misregulation of MAP kinases activities, over accumulation of the defense hormone salicylic acid and transcriptional reprogramming of defense and cell death regulatory genes. Furthermore, *OXI1* over expressing lines display increased disease resistance to *Pseudomonas syringae* bacteria and altered pathogen-induced cell death programs. In sum our results suggest that, similarly to the function of AGC kinases in animals, the *OXI1* kinase plays an important role in controlling plant growth, disease resistance and cell death programs in *Arabidopsis*.

Keywords: *OXI1* kinase, PDK1, TOR, cell death

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Session 4
Socio-economic issues
related to the use of resistant
varieties
and their deployment in agro-
systems

The farmers view about the use of different agricultural crop varieties in the federal capital territory Abuja Nigeria

Adedotun M.

Abstract

Agriculture is the engine room of any country, because of the important it has in the developmental process. Many farmers in the Federal Capital Territory, use to engage in Agricultural practices such as planting of different varieties of crops and rearing of cattles. These crops are used to field different cattles. Different Pictures were taken to ascertain the different varieties of plants such as Maize, Yam and Groudnut being cultivated by the farmers. And the different varieties of crops in which cattle feed on for growth and productivitiy and Farm and Home Visits were also made to get the various informations. All this give the vital Information in this paper.

Keywords: Different crops, varieties, farmers