Book of Abstracts

COST Action SUSTAIN

FA1208

Workshop

Pathogen-Informed Crop Improvement

8 - 10 April 2015,

Hotel De Wageningsche Berg,

Wageningen, the Netherlands

Scientific committee
Thomas Kroj
Eva Stukenbrock
Susan Gabriels
Vivianne Vleeshouwers
Bart Thomma
Beat Keller
Gregori Bonnet
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Aska Goverse

Local organization
Aska Goverse and Christel van Geelen
Laboratory of Nematology, Wageningen University, the Netherlands

Meeting overview

Wednesday 8th April	Thursday 9th April	Friday 10th April
	9.00 – 10.10 ***Session 3*** ***Effector-breeding: promises and limitations II***	9.00 – 10.10 ***Session 7*** ***Exploitation of novel resistance sources and strategies I***
	10.15– 10.45 **Coffee Break**	10.15 – 10.45 **Coffee Break**
	10.45 – 12.05 ***Session 4*** ***Effector diversity and host adaptation: virulence spectra II & Discussion***	10.45 – 12.15 ***Session 8*** ***Exploitation of novel resistance sources and strategies II & Discussion***
13.00 – 13.45 **Registration / Welcome**	12.15 – 13.15 **Lunch** 13.15 – 14.30 **WALK ARBORETUM WAGENINGSE BERG**	12.15 – 12.30 **Concluding remarks and closure** 12.30 – 13.30 **Lunch**
13.45 – 15.25 ***Session 1*** ***Effector diversity and host adaptation: virulence spectra I***	14.45 – 16.00 ***Session 5*** ***Durable host resistance: R and S genes I***	
15.30 – 16.00 Cofee Break	16.00 – 16.30 **Refreshments**	
16.00 – 18.45 ***Session 2*** ***Effector-breeding: promises and limitations I & Discussion***	16.30 – 18.10 ***Session 5*** ***Durable host resistance: R and S genes II & Discussion***	
19.00 – 21.30 **Conference Dinner**	18.30 – 19.30 **Dinner**	
	19.45 – 21.00 ***Debate Session***	

Wednesday April 8th

13.00 Registration
13.30 Welcome Aska Goverse and Thomas Kroj

Session 1 - Effector diversity and host adaptation: virulence spectra I

Chairs: Eva Stukenbrock and Suzan Gabriels

13.45 – 14.15 **Thierry Rouxel**
(INRA, BIOGER, Thiverval-Grignon, France)
Diversity of evolutionary mechanisms leading to virulence when Leptosphaeria maculans is faced to a Brassica resistance gene

14.15 – 14.35 **Jari Valkonen**
(University of Helsinki, Finland)
Resistance gene *Ny* in potato recognizes the RNAi suppressor HCpro, an effector of *Potato virus Y*

14.35 – 14.55 **Thomas Kroj**
(INRA, Montpellier, France)
Effector content governs local adaptation of the rice blast fungus

14.55 – 15.25 ***Bruce McDonald***
(ETH Zurich, Switzerland)
Breeding Strategies Based on Dynamic Diversity to Achieve Durable Disease Resistance

15.30 – 16.00 COFFEE/TEA BREAK

Session 2 - Effector-breeding: promises and limitations I

Chairs: Vivianne Vleeshouwers and Thomas Kroj

16.00 – 16.30 **Vivianne Vleeshouwers**
(Laboratory of plant breeding, Wageningen University, The Netherlands)
Effector-assisted breeding for disease resistance in potato

16.30 – 16.50	**Amir Mirzadi Gohari** ***(Wageningen University, The Netherlands)*** Effector discovery in the fungal wheat pathogen *Zymoseptoria tritici*
16.50 – 17.10	**Marc-Henri Lebrun** ***(INRA, Biologie Gestion des Risques en agriculture Thiverval-Grignon, FRANCE)*** Wheat Effector Assisted Breeding for Resistance to Fungal Pathogens
17.15 – 17.45	GENERAL DISCUSSION SESSION 1 AND 2
17.45 – 18.45	DRINKS AND INFORMAL DISCUSSIONS
19.00 - 21.30	CONFERENCE DINNER

Thursday April 9th

Session 3 - Effector-breeding: promises and limitations II

Chairs: Eva Stukenbrock and Suzan Gabriels

9.00 – 9.30	**James Brown** **(*John Innes Centre, Norwich, UK*)** Predicting Durability of Disease Resistance
9.30 – 9.50	**Eva Holtgrewe Stukenbrock** ***(Christian-Albrechts University of Kiel, Germany)*** Expression profiling of the wheat pathogen Zymoseptoria tritici reveals genomic patterns of transcription and host-specific regulatory programs
09.50 – 10.10	**Martijn Rep** ***(University of Amsterdam, The Netherlands)*** Effector gene content in *Fusarium oxysporum* predicts host-specific pathogenicity
10.15 - 10.45	COFFEE/TEA BREAK

Session 4 - Effector diversity and host adaptation: virulence spectra II

Chairs: Vivianne Vleeshouwers and Thomas Kroj

10.45 – 11.15	**Richard Oliver** **(*Curtin University, Perth, Australia*)** Exploitation of necrotrophic effectors to improve crop protection
11.15 – 11.35	**Ulrich Schaffrath** ***(RWTH Aachen University, Germany)*** Comparative analysis of Candidate Effector Genes in different Magnaporthe species
11.35 – 12.05	GENERAL DISCUSSION SESSION 3 AND 4
12.15 – 13.15	LUNCH

13.15 – 14.30 WALK ARBORETUM WAGENINGSE BERG

14.30 – 14.45 TEA/COFFEE BREAK

Session 5 - Durable host resistance: R and S genes I

Chairs: Bart Thomma and Gregori Bonnet

14.45 – 15.15 **Yuling Bai**
(Wageningen University, The Netherlands)
Breeding crops with disease resistance by editing plant susceptibility genes

15.15 – 15.35 **Cyrille Saintenac**
(INRA, Génétique, Diversité, Ecophysiologie des Céréales, Clermont-Ferrand, France)
Fine mapping of *Stb16q*, a major resistance gene effective against *Zymoseptoria tritici* in wheat

15.35 – 15.55 **Yin Song**
(Laboratory of Phytopathology, Wageningen University, The Netherlands)
Are tomato Ve1 homologs ancient immune receptors that are conserved across the plant kingdom

16.00 - 16.30 REFRESHMENTS

Session 6 - Durable host resistance: R and S genes II

Chairs: Bart Thomma and Gregori Bonnet

16.30 – 17.00 **Guido van der Ackerveken**
(Utrecht University, The Netherlands)
Host genes affecting plant susceptibility to oomycetes

17.00 – 17.20 **Liliya Pylypenko**
(Institute of Plant Protection of National Academy of Agrarian Sciences of Ukraine, Ukraine)
Polymorphism of the DNA markers of Resistance associated genes among Ukrainian common wheat and potato cultivars

17.20 – 17.40	**<u>Geert Smant</u>** **(Laboratory of Nematology, Wageningen University, The Netherlands)** Breeding natural nematode resistant crops by quantitative loss of susceptibility
17.40 – 18.10	GENERAL DISCUSSION SESSION 5 AND 6
18.30 – 19.30	BUSINESS DINNER
19.30 – 19.45	COFFEE/TEA BREAK
19.45 ~ 21.00	DEBATE

Friday April 10th

Session 7 - Exploitation of novel resistance sources and strategies I

Chairs: Aska Goverse and Beat Keller

09.00 – 09.30 **Ingo Hein**
(James Hutton Institute, Dundee, UK)
Target enrichment and next generation sequencing in potato to accelerate the mapping and cloning of disease resistance gene

09.30 – 09.50 **Johanna Acevedo-Garcia**
(RWTH Aachen University. Institute for Biology I. Unit of Plant Molecular Cell Biology, Germany)
Exploitation of *mlo*-based broad-spectrum powdery mildew resistance in wheat

09.50 – 10.10 **Hélène Missionnier**
(Syngenta France SAS, Saint Sauveur, France)
Sunflower-Pathogen Field Interaction: Spatial Patterns of the Disease Using Geostatistics Applied to Fixed Replicated Controls

10.15 – 10.45 TEA/COFFEE BREAK

Session 8 - Exploitation of novel resistance sources and strategies I

Chairs: Aska Goverse and Beat Keller

10.45 – 11.15 **Pim Lindhout**
(Solynta, Wageningen, The Netherlands)
Recent developments in science and breeding to generate hybrid potato varities with durable resistance to Phytophthora infestans

11.15 – 11.45 **Inger Ahman**
(Department of Plant Breeding, Swedish University of Agricultural Sciences, Sweden)
Two examples where site-directed mutagenesis is attempted as a resistance breeding method; for aphid and net blotch resistance

11.45	GENERAL DISCUSSION SESSION 7 AND 8
12.15	**Concluding remarks and closure**
12.30	LUNCH + DEPARTURE

Statements for Discussion in 5 thematic groups (Debate day2)

You can sign in for one of the thematic areas during the workshop

Theme: "effectors" I

1) Studying effectors is a must to understand plant-pathogen interactions, but it does not contribute to the implementation of durable/quantitative resistance in practice.
2) A standard protocol to identify functional effectors for each pathogen of interest will enable all breeding companies to perform effector based screenings on breeding material or R sources.

Theme: "effectors" II

3) Understanding the population dynamic of pathogens and the evolution of effector genes is essential in the development of durable resistances.
4) Collaboration via public-private partnerships is currently used as an instrument to stimulate knowledge transfer. Are there more effective ways to transfer knowledge between academia and industry?

Theme: "Durable host resistance"

1) If the S protein is a target for a crucial pathogen-effector, targeted modification of *S* genes is the solution for durable and broad resistance.
2) In 20 years, growing many crops in quarantine conditions in flats with led-lights will reduce the need for plants with resistance to pathogens.
3) What are our wishes/dreams (tools/ outcomes/opportunities) for the long term future (20 years?)

Theme: "Pre-breeding"

1) Mapping of *R* genes via transient expression or infiltration of effectors is more effective and high-throughput than via regular disease assays; however "R-Avr recognition" is not representative for the "R-pathogen recognition", and can therefore be misleading in pre-breeding.
2) In 20 years, breeding for resistance will be selection of resistant plants by Genome-Wide-Association studies.
3) In the future plants are designed for bio refinery purposes and food ingredient production.

Theme: "Exploitation of novel resistant sources"

1) Identification of novel sources by effector-based screening will generate "misleading" sources, recognizing only 1 effector instead of resistance to the pathogen (with lots of effectors).
2) In the future, a shortage of nutrients will be limiting plant production, rather than plant defence against pathogens.

Abstracts

Session 1

Effector diversity and host adaptation: virulence spectra I

Chairs: Eva Stukenbrock and Suzan Gabriels

Diversity of evolutionary mechanisms leading to virulence when *Leptosphaeria maculans* is faced to a Brassica resistance gene

Thierry Rouxel, Clémence Plissonneau, Isabelle Fudal, Marie-Hélène Balesdent

INRA, UMR INRA-APT Bioger, av. Lucien Brétignières, 78850 Thiverval-Grignon, France

(Missing)

Resistance gene *Ny* in potato recognizes the RNAi suppressor HCpro, an effector of *Potato virus Y*

Y.P. Tian and Jari.P.T. Valkonen

University of Helsinki, Finland

Potato virus Y (PVY) is the most economically damaging and widely distributed virus in potato. Spread of PVY in the field is controlled by growing resistant cultivars. The dominant gene Ny_{tbr} for hypersensitive resistance (HR) controls ordinary PVY strains (PVY^O) in potato but is overcome by PVY^N strains. Studies with infectious PVY chimeras and mutants indicated that the viral determinants necessary and sufficient to overcome Ny_{tbr} reside within the helper component proteinase (HCpro residues 227–327). Specifically, eight residues and the modelled three-dimensional conformation of this HCpro region distinguish PVY^N from PVY^O strains. Two residues (R269, K270) are crucial for the predicted PVY^O-specific HCpro conformation. Our results suggest a structure-function relationship in recognition of PVY^O HCpro by Ny_{tbr}, reveal HCpro amino acid signatures specific to PVY^O and PVY^N, and facilitate identification of PVY strains overcoming Ny_{tbr}. Furthermore, our study indicates that Ny_{tbr} recognizes an important virulence determinant (effector) of PVY implicated in suppression of RNA silencing, the fundamental, basal antiviral defence mechanism in plants. Hence, HCpro-*Nytbr* interaction represents an example of virus–*R* gene interactions that is consistent with theories suggesting that *R* genes have evolved to recognize important basal defence suppressors (effectors) produced by plant pathogens.

References: Tian & Valkonen 2013, MPMI 26: 297–305; Tian & Valkonen 2015, Mol. Plant Pathol 16, in press)

Effector content governs local adaptation of the rice blast fungus

Liao J[1,2,3], Huang H[1,2,3], Meusnier I[1,2], He X[1,2,3], Tharreau D[1,2], Fournier E[1,2], Kroj T[1,2], Morel JB[1,2]

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Scarce cases of durable disease resistance have been documented in plant/pathogen systems. Their thorough analysis may help to understand how durable resistance emerges and is maintained and how it can be exploited in a sustainable manner. We have initiated the analysis of durable resistance of rice to blast disease caused by the fungus *Magnaporthe oryzae* in the Yuanyang terraces in the Yunnan province in China. Multi-year sampling of fungal isolates on glutinous rice and non-glutinous rice indicate that two populations of the blast fungus co-exist and are only rarely exchanged between these two rice hosts. Evaluation of the number of avirulence (Avr) effectors in the two *M. oryzae* subpopulations demonstrate that isolates from glutinous rice possess particularly high numbers of Avr effectors suggesting that the population from glutinous rice is specialized on its host. Moreover, aggressiveness of these isolates on glutinous rice and non-glutinous rice varieties is correlated with the Avr effector content. Preliminary results from experiments with isogenic *M. oryzae* strains pinpoint one Avr effector that seems to play a key role in the local adaptation of the two blast subpopulations.

Breeding Strategies Based on Dynamic Diversity to Achieve Durable Disease Resistance

Bruce A. McDonald

Plant Pathology Group, ETH Zurich, Switzerland

Disease resistance fails because pathogens evolve. Evolution requires genetic diversity. Genetic diversity is affected by mutation, population size, recombination, gene flow, and selection, the same factors that affect an organism's population genetics. Thus the keys to developing strategies for breeding durable disease resistance lie in understanding pathogen population genetics. Truly durable resistance (i.e. highly effective resistance that prevents a damaging epidemic over temporal scales measured in decades or centuries and spatial scales measured in tens of thousands or millions of hectares) will not be achieved unless we increase the overall diversity present in agroecosystems at both the farm and landscape spatial scales. The diversity will need to be dynamic, changing regularly over both time and space, to significantly slow the rate of pathogen adaptation. How can we increase total agroecosystem diversity at the farm and landscape scale without losing the advantages that come with increasing crop uniformity? Fortunately, a wide array of low-technology, medium-technology and high-technology strategies can be used to increase both spatial and temporal diversity in agroecosystems. The resistance gene cassette represents the highest currently available technology.

Session 2

Effector-breeding: promises and limitations I

Chairs: Vivianne Vleeshouwers and Thomas Kroj

Effector-assisted breeding for disease resistance in potato

Vivianne G. A. A. Vleeshouwers, Juan Du, Emmanouil Domazakis, Xiao Lin, Carolina Galvez Aguilera, Gerard Bijsterbosch, Doret Wouters, Evert Jacobsen, Richard G.F. Visser

Wageningen UR Plant Breeding, Wageningen University and Research Centre, Droevendaalsesteeg 1, 6708 PB, Wageningen, The Netherlands

Since the genomics era, effectors are emerging as tools to accelerate and improve the identification, functional characterisation and deployment of disease resistance genes. In recent years, the effectoromics strategy has proven effective and complementary to classical breeding for resistance to the potato late blight pathogen *Phytophthora infestans*. We have generated a genome-wide infection-ready library of *P. infestans* RXLR effectors that include avirulence (AVR) proteins, which are targeted by resistance (R) proteins. This has accelerated cloning and specificity profiling of *R* genes from wild potato species. Studies of effector diversity and activity revealed the mechanisms that *P. infestans* employs for evading R protein recognition for the various R-AVR pairs. Spatio-temporal monitoring of effector allelic diversity in *P. infestans* populations enables a more educated deployment of *R* genes in potato. Recently, we have expanded the R-AVR-based line of defense with studies on apoplastic immunity, which has generally a broader spectrum and is based on recognition of conserved proteins of pathogens. We have isolated a potato surface receptor ELR that senses elicitins, secreted oomycete proteins with features of pathogen-associated molecular patterns (PAMP). In transgenic potatoes, *ELR* confers a hypersensitive response to INF1 elicitin of *P. infestans* and enhanced resistance to late blight. Our aim is to achieve effective and more durable resistance against late blight in potato by combining multiple layers of immunity.

Effector discovery in the fungal wheat pathogen *Zymoseptoria tritici*

Amir Mirzadi Gohari[1,2], Sarah B. Ware[1], Alexander H.J. Wittenberg[3], Rahim Mehrabi[4], Sarrah Ben M'Barek[1,5], Els C.P. Verstappen[1], Theo A.J. van der Lee[1], Olivier Robert[6], Henk J. Schouten[3], Pierre P.J.G.M. de Wit[7], Gert HJ Kema[1]

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Fungal plant pathogens such as *Zymoseptoria tritici* (formerly known as *Mycosphaerella graminicola*) secrete repertoires of effectors facilitating infection or triggering host defence mechanisms. Discovery and functional characterization of effectors renders valuable knowledge that contributes to designing new and effective disease management strategies. Here, we combined bioinformatics approaches with expression profiling during pathogenesis to identify candidate effectors of *Z. tritici*. Additionally, a genetic approach was conducted to map quantitative trait loci (QTL) carrying putative effectors enabling the validation of both complementary strategies for effector discovery. *In planta* expression profiling revealed that candidate effectors were up-regulated in successive waves corresponding with consecutive stages of pathogenesis, contrary to candidates identified by QTL mapping that were overall lowly expressed. Functional analyses of two top candidate effectors (SSP15 and SSP18) showed their dispensability for *Z. tritici* pathogenesis. These analyses reveal that generally adopted criteria such as protein size, cysteine residues and expression during pathogenesis may preclude an unbiased effector discovery. Indeed, genetic mapping of genomic regions involved in specificity render alternative effector candidates that do not match the aforementioned criteria, but should nevertheless be considered as promising new leads for effectors that are crucial for the *Z. tritici*-wheat pathosystem.

Wheat Effector Assisted Breeding for Resistance to Fungal Pathogens (WEAB)

Marc-Henri Lebrun[1], Thierry Langin[2], Thomas Kroj[3], James Cockram[4], Richard Oliver[5], Gert Kema[6], Romain Valade[7], Sébastian Praud[8], Valérie Laurent[9], Laure Duchalais[10], Volker Lein[11]

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The discovery that fungal effector proteins are important for infection represents a novel opportunity for controlling crop diseases. Use of fungal effectors for resistance breeding is a game-changing technology that will create opportunities and innovative methods to identify novel plant resistances. These methods are amenable to high throughput screening facilitating the detection and introduction of novel resistance genes in elite cultivars. The objective of WEAB is to use toxic protein effectors from four main fungal pathogens of wheat, *Fusarium graminearum (Fg), Mycosphaerella graminicola (Mg), Pyrenophora tritici-repentis (Ptr)* and *Parastagonodorum nodorum (Sn)* to detect novel wheat resistance genes/QTLs. These aims are greatly complemented by the recent availability of high-density genetic marker coverage of the wheat genome. Complementary strategies will be used to detect a large array of resistance mechanisms to fungal effectors. Type I strategy relies on delivering recombinant toxic protein effectors into wheat leaves. The project will focus on type I assays using fungal toxic effectors that are either known (Ptr, Sn) or suspected (Mg, Fg). Type I assays will be used to screen elite wheat cultivar collections previously genotyped with SNP arrays. This will lead to the identification of cultivars resistant to these toxins and the mapping of corresponding loci using genome-wide association analyses. Type II strategy is based on the identification of wheat effector targets

(WET) using molecular methods for the detection of protein-protein interactions. WET genes could be localized on wheat genome allowing their mapping relative to known QTLs conferring resistance to fungal pathogens. This project will facilitate plant breeding efforts to select for resistance to important fungal pathogens by providing a 'toolkit' of bio-molecular markers.

Session 3

Effector-breeding: promises and limitations II

Chairs: Eva Stukenbrock and Suzan Gabriels

Predicting Durability of Disease Resistance

James K M Brown

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There is no single genetic or phenotypic model for durable resistance. Nevertheless, an understanding of coevolution between parasites and their hosts allows predictions about when resistance is more likely to be durable. The reciprocal nature of host-parasite interactions means that long-term frequencies of parasite avirulence genes depend on fitness costs acting on the host and, conversely, that long-term frequencies of resistance genes depend on fitness costs in the parasite. Resistance is predicted to be more common over the long term if the cost of virulence is high. An indirect prediction is that a resistance gene with a high frequency in nature may be durable in agriculture because the corresponding virulence may be costly. Durability of resistance is increased if disease levels are kept low, for example by the use of partial, 'background' resistance or by effective pesticide regimes. Other predictors of durability are that the resistance gene has low specificity against avirulent parasites and
that resistance is costly, which causes the long-term frequency of avirulence to be high; neither of these options seems particularly attractive for commercial plant breeding. If a virulence gene is rare, there is no reason to suppose that the corresponding resistance is necessarily durable; rather, it implies either that resistance is costly or that the disease is unimportant in the population studied. A goal for future research is to understand why resistance gene 'pyramids' are sometimes durable but often are not; the most plausible explanation for durability is that there is a synergistic cost of virulence. Another important challenge is to understand the interaction between the evolution of plant resistance genes and the community dynamics of different pathogens. While it is now possible to predict which kinds of resistance are more likely to be durable, a stable equilibrium between plants and their parasites can only evolve and resistance can only be truly durable if there are environmental factors which weaken the inter-dependence of the host and parasite's life-cycles and thus generate direct frequency-dependent selection. Many of these factors are present in nature but have been intentionally removed from agriculture, especially from modern industrial farming. A long-term future for food production may involve a trade-off between productivity and environmental complexity.

Expression profiling of the wheat pathogen Zymoseptoria tritici reveals genomic patterns of transcription and host-specific regulatory programs

Ronny Kellner[1], Amitava Bhattacharyya[1], Stephan Poppe[1], Tiffany Y. Hsu[2] , Rachel B. Brem[2] and Eva H. Stukenbrock[1,3]

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Host specialization by pathogens requires a repertoire of virulence factors and fine-tuned regulation of gene expression. The fungal wheat pathogen *Zymoseptoria tritici* (synonym *Mycosphaerella graminicola*) is a powerful model system for the discovery of genetic elements that underlie virulence and host specialization. We analyzed early stages of *Z. tritici* infection of a compatible host (wheat) and a non-compatible host, the grass species *Brachypodium distachyon*. The results revealed infection regulatory programs common to both hosts as well as genes with striking wheat-specific expression, with many of the latter showing sequence signatures of positive selection in *Z. tritici*. Genes specifically regulated during infection of wheat included two large clusters of co-regulated genes that may represent candidate pathogenicity islands. On the repeat-rich accessory chromosomes, we identified hundreds of highly expressed genes with signatures of evolutionary constraint and putative biological function. Phylogenetic analyses suggested that gene duplication events on these accessory chromosomes were rare and largely preceded the diversification of *Zymoseptoria* species. Together, our data highlight the likely relevance for fungal growth and virulence of hundreds of *Z. tritici* genes, deepening the annotation and functional inference of the genes of this model pathogen.

Effector gene content in *Fusarium oxysporum* predicts host-specific pathogenicity

Martijn Rep

Molecular Plant Pathology, Swammerdam Institute for Life Sciences, University of Amsterdam, The Netherlands

Fusarium oxysporum is a global fungal species complex harbouring largely non-pathogenic strains. However, clonal lines have emerged from this species complex that cause wilt disease in many important vegetable and flower crops, such as tomato, pea, melon, banana, strawberry, cotton and carnation. Four clonal lines are known that cause wilt disease in tomato. These four lines are polyphyletic within the species complex but they still produce largely the same set of fourteen effectors (called Six1-14) during infection of tomato; only one or two effectors are absent from some strains and only in two effectors a single nucleotide polymorphism has been found. This can be explained by our finding that the genes for these effectors reside on a single chromosome that can be horizontally transferred between strains. The four clonal lines that infect tomato have apparently emerged from horizontal chromosome/effector gene transfer. To investigate the extent to which this may hold true for other host-specific forms, we have obtained genome sequences from 30 strains infecting melon, cucumber and/or watermelon as well as *in vitro* and *in planta* expressed sequences. We determined the effector content of these strains as well as of strains for which genome sequences have been made available by the Broad Institute. When we group all strains based on effector content, strains infecting the same host cluster together, despite their polyphyletic nature. We conclude that we can use effector content to predict host-specific pathogenicity of strains of *Fusarium oxysporum*.

Session 4

Effector diversity and host adaptation: virulence spectra II

Chairs: Vivianne Vleeshouwers and Thomas Kroj

Exploitation of necrotrophic effectors to improve crop protection

Richard Oliver, Huyen Phan, Kar-Chun Tan, Kasia Rybak, Caroline Moffat, Pao Theen See.

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The role of necrotrophic effectors in promoting virulence can be exploited as a way to select more resistant germplasm. Resistance to necrotrophic diseases was typically found to be partial, in contrast to the major gene resistance noted in some cases for biotrophic pathogens. This has meant that breeding for disease resistance is much more demanding and explains why necrotrophic pathogens have grown in importance whilst progress in controlling biotrophic diseases was often rapid (until the emergence of the next mutant pathogen race). However the identification and production of cloned and expressed effectors of necrotrophic pathogens allows breeders to select introgressions that are insensitive. Effectors from both Parastagonospora nodorum and Pyrenophora tritici-repentis have been ex-pressed in microbial systems and used to identify germplasm that is insensitive to the effector. Thus, in the case of multi-effector systems like P. tritici-repentis and P. nodorum, selection of cultivars insensitive to each effector promises to assists breeders improve disease resistance in an incremental, step-wise fashion. Progress and complications in this process will be discussed and reviewed.

Comparative analysis of Candidate Effector Genes in different *Magnaporthe* species

Valerie Mogga[1], Rhoda Delventhal[1], Philipp M. Bertram[1], Thomas Kroj[2] and Ulrich Schaffrath[1]

[1] Department of Plant Physiology, RWTH Aachen University, D-52074 Aachen, Germany
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While isolates of *Magnaporthe oryzae* establish a host interaction with barley or wheat, isolates of *Magnaporthe grisea* which are virulent on *Digitaria sanguinalis* establish a nonhost interaction with both crop plants (Zellerhoff *et al.*, 2006). Transcript profiling in barley epidermal peels after inoculation with host- or nonhost *Magnaporthe* isolates led to a list of hypothetical effector genes (*HEGs*) which accumulated to a higher degree in the host compared to the nonhost interaction. Homologues of *M. oryzae HEGs* were identified in *M. grisea* and expression profiles are in most cases conserved among *HEGs* from both species.

Nep1 (**n**ecrosis- and **e**thylene-inducing **p**rotein 1)-like proteins (NLPs) are highly conserved among pathogenic bacteria, fungi and oomycetes and are believed to act as toxins rather than elicitors in dicotyledonous plants. Although there are no dicotyledonous hosts known for *Magnaporthe spec*., isolates of *M. oryzae* contain four *NLP* genes (*MoNLPs*) and two of them were expressed during colonization of barley. *Agrobacterium*-infiltration assays revealed the capacity of three MoNLPs to induce cell death in tobacco. This cell death could be suppressed by co-expression of the *Colletotrichum higginsianum (Ch)* effector candidate 3 (ChEC3) that suppresses ChNLP1 induce cell death as well (Kleemann *et al.*, 2012). Remarkably, also one MoHEG counterattacked the MoNLP induced cell death response in tobacco. The conservation of this corresponding pair of *M. oryzae* effectors may point to a so far undetected function of NLPs in the life-cycle of this monocot pathogen.

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Session 5

Durable host resistance: R and S genes I

Chairs: Bart Thomma and Gregori Bonnet

Breeding crops with disease resistance by editing plant susceptibility genes

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Plants are continuously attacked by a broad range of pathogens. World-wide farmers use large amounts of chemicals to secure crop yield. Breeding for disease resistance is a major objective of breeding activities in order to minimize the yield and quality loss associated with disease. Although, resistance can often be obtained by introgression of major resistance genes (*R*-genes) from wild crop relatives, resistance conferred by *R*-genes is rarely durable. In Arabidopsis, increased research with a focus on suppression of plant immunity has led to the identification of disease-susceptibility genes (*S*-genes). The *S*-gene is a plant gene required for pathogen survival and proliferation, which was first highlighted in 2002 by Eckardt when *Pmr6* was discovered in Arabidopsis as a gene coding for a susceptibility factor to promote growth of powdery mildew. However, large scale *S*gene identification has not been performed in crops yet. In 2011, we proposed to use *S*-genes in breeding crops for resistance to pathogens. In the last few years, we have carried out proof-of-concept research. We selected tomato/potato orthologs corresponding to about 20 Arabidopsis *S*genes. Using RNAi, our results showed that silencing certain *S*-genes resulted in complete resistance to powdery mildew and late blight in both tomato and potato. Thus, our research illustrates that the *S*-genes identified in Arabidopsis are conserved for their function as susceptibility factors in other plant species. In complementation to the introgression of *R*-genes, durable and broad-spectrum resistance can be achieved by editing plant *S*-genes.

Fine mapping of *Stb16q*, a major resistance gene effective against *Zymoseptoria tritici* in wheat

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Septoria tritici blotch (STB) caused by the fungus *Zymoseptoria tritici* (formerly called *Mycosphaerella graminicola*) is the most damaging wheat disease in Europe. So far, nearly twenty major disease resistance genes (named '*Stb'*) have been identified and genetically mapped. Most of these confer isolate-specific resistance. Only *Stb16q* shows resistance to all isolates tested. This gene, which was discovered in a synthetic hexaploid line, controlled resistance to necrotic leaf area, leaf-bearing pycnidia and latent period. Toward functional characterization, we initiated the fine mapping of *Stb16q* in wheat using a large F2 mapping population derived from a cross between TA4152-19 (*Stb16q*) and the fully susceptible line ND495. *Stb16q* mapped to the long arm of chromosome 3D and behaved as a dominant character in this population. Using bin-mapped ESTs, markers from high density reference maps and the D-genome physical map, we delineated *Stb16q* to a 0.14 cM interval. In addition, we identified several markers that co-segregate with *Stb16q*. Screening a TA4152-19 BAC library with those markers led to the identification of a positive BAC clone. Analysis of the BAC sequence revealed the presence of a cluster of receptor-like kinase genes (RLKs). These genes represent good candidates for *Stb16q* to derive potentially durable resistance against this devastating disease.

Are tomato Ve1 homologs ancient immune receptors that are conserved across the plant kingdom?

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Pattern recognition receptors (PRRs) confer plant resistance to pathogen infection by recognizing conserved microbe-associated molecular patterns (MAMPs). The cell surface-localized receptor tomato Ve1 confers racespecific resistance against *Verticillium dahliae* by recognizing the effector Ave1 (for Avirulence on Ve1 tomato) that is secreted by race 1 strains of the soil-borne vascular wilt fungus *V. dahliae*. In addition, Ave1 homologs were found in plants and microbes, and these homologs are differentially recognized by tomato Ve1.The demonstration of interfamily transfer of tomato *Ve1* to the phylogenetically distant species Arabidopsis implies evolutionary conservation of the underlying immune signaling cascade across plant taxonomy. Although several Ve1 homologs were identified within and outside the *Solanaceae* family, functionality of these homologs as immune receptor against *Verticillium* infection often lacks. Here, we describe the cloning and characterization of *Verticillium* wilt resistance genes from *Nicotiana glutinosa* and *Humulus lupulus,* and demonstrate that these homologs govern resistance against *Verticillium* race 1 strains through recognition of the Ave1 effector. Phylogenic analysis shows that Ve1 homologs are widely distributed in land plants. Our study unravels that tomato Ve1 homologs are ancestral immune receptors that are conserved across the land plant kingdom.

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Session 6

Durable host resistance: R and S genes II

Chairs: Bart Thomma and Gregori Bonnet

Host genes affecting plant susceptibility to oomycetes

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Oomycetes constitute an important group of plant pathogens. Our work focuses on the downy mildews that are obligate biotrophic pathogens that affect the production of many crops. To understand the molecular processes underlying host plant susceptibility to downy mildews we have taken two main approaches. Our genetic approach has resulted in the identification of *DMR* (*DOWNY MILDEW RESISTANT*) genes. I will report on our advances on the function of the oxygenase DMR6 and related oxygenases of Arabidopsis, that act as negative regulators of immunity. A second approach makes use of the effectors of downy mildews. Genome analysis of different downy mildew species has resulted in the identification of hundreds of effector candidates. Functional studies and yeast-two hybrid experiments have revealed host proteins that are targeted by effector proteins. These host targets are of interest as they have the potential to be used in breeding new downy mildew resistant crops.

Polymorphism of the DNA markers of resistance associated genes among Ukrainian common wheat and potato cultivars

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One hundred and sixty cultivars of common wheat (*Triticum aestivum* L.) of Ukrainian breeding were studied using molecular genetic markers of the *Tsn1* gene associated with sensitivity to the *Pyrenophora tritici-repentis* and *Stagonospora nodorum* toxins A, the *Tsc2* gene associated with sensitivity to the *P. tritici-repentis* toxin B and the *Lr34/Yr18/Pm38/Sr57/Bdv1* gene associated with multiple adult plant resistance. The frequency of the ToxA insensitivity allele of the *Tsn1* gene marker made up 60% and the frequency of the ToxB insensitivity allele of the *Tsc2* gene marker made up 45%. The frequency of the resistance alleles of the *Lr34* gene markers made up 51.2% (including polymorphic cultivars). It is higher than that for the wheat germplasm of the USA, Great Britain, Australia, India and Canada according to literature. So the Ukrainian wheat cultivars are not only the ones with the highest baking quality and yield characteristics but also carry important factors of resistance against multiple pathogens. Forty cultivars of potato (*Solanum tuberosum* L.) of Ukrainian breeding were studied using molecular genetic markers TG689 and 57R, linked to *H1* gene associated with the resistance against *Globodera rostochiensis*, revealing a high level of coincidence of the presence of marker alleles with phenotypic resistance of potato cultivars – 80 and 95 % respectively. It is proposed to introduce these molecular markers in the breeding process of wheat and potato in order to simplify and accelerate the sampling of genotypes carrying the target alleles of relevant genes. There is a need to use such an approach in soya been (*Glycine max* (L.) Merr.) and sugar beet (*Beta vulgaris* L.) breeding programs for better utilization of genetic sources for *Heterodera glycines* and *Heterodera schachtii* resistance respectively, presented in national gene bank collection.

Breeding natural nematode resistant crops by quantitative loss of susceptibility

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Sedentary plant-parasitic nematodes modify host cells into permanent feeding structures. The capacity of these permanent feeding structures to continuously deliver nutrients to feeding nematodes for several weeks is a crucial factor for nematode propagation on plants. The ontogeny of nematode-induced feeding structures involves hundreds of different plant genes. Plants are thought to harbour quantitative genetic variation in their ability to facilitate the flow of nutrients via the nematode-induced feeding structures. But, the underlying molecular basis of this quantitative variation in susceptibility is not well understood. We have recently shown that natural allelic variation in plant genes that are essential for parasitism of nematodes can have a significant quantitative impact on nematode propagation. Our findings demonstrate that breeders can exploit this natural variation for breeding nematode resistant crops by accumulating multiple loss-of-susceptibility alleles in their germplasm.

Session 7
Exploitation of novel resistance sources and strategies I

Chairs: Aska Goverse and Beat Keller

Target enrichment and next generation sequencing in potato to accelerate the mapping and cloning of disease resistance genes

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The majority of cloned and functional disease resistance (*R*) genes identified within the plant kingdom contain nucleotide-binding (NB) and leucine-rich repeat (LRR) domains. NB-LRR genes are implicated in resistances against diverse and taxonomically unrelated pathogens including bacteria, viruses, nematodes, insects, filamentous fungi and oomycetes.

An extensive annotation of the NB-LRR-type genes in potato has provided a blueprint for the more rapid mapping and cloning of functional resistance genes. We have developed RenSeq (Resistance gene enrichment and Sequencing) and the accompanying bioinformatics tools to map resistances in bulked segregating populations. This approach has been used successfully to map a novel resistance to the late blight pathogen *Phytophthora infestans* in the Mexican diploid potato species *Solanum verrucosum*.

A BC1 segregating population derived from an intraspecies cross between a homozygous resistance clone (VER54/15) and a susceptible clone (VER3939/17) was constructed for the genetic study of the underlying resistance. Previous efforts to identify the resistance locus with traditional markers such as AFLP were hampered by a relatively low level of polymorphism within the population. RenSeq analysis identified informative single nucleotide polymorphisms (SNPs) and delimited a target region on linkage group 9. Further efforts to develop sequencing-based markers combined with genotyping and phenotyping of an enlarged segregating population have enabled the fine mapping of the resistance.

***Breeding strategies*: Exploitation of *mlo*-based broad-spectrum powdery mildew resistance in wheat**

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With more than 713 million tonnes produced in 2013, bread wheat (*Triticum aestivum*) is the third grown crop in the world and the second in terms of dietary intake. To meet the demand of a growing population, wheat breeders and growers have the challenge to increase the wheat production by around 60% by 2050. To contribute to this task we are working on the generation of wheat plants resistant to powdery mildew, one of the diseases that severely affect yield. We exploit the *mlo*-based resistance and the non-transgenic technology TILLING (Targeted Induced Local Lesions IN Genomes) for this purpose. Loss-of-function alleles of *Mlo* (*Mildew resistance locus o*) gene(s) in barley, tomato, pea and Arabidopsis confer non-race specific resistance to the fungal pathogens that cause powdery mildew disease in these species. In allohexaploid wheat, three orthologs (wheat homoeologs) of barley *Mlo* (*TaMlo-1A*, *TaMlo-1B*, *TaMlo-1D*) have been identified. Using TILLING in a mutagenized population of wheat cultivar Cadenza we found mutations in the form of single nucleotide polymorphisms in the three *Mlo* homoeologs. Several missense mutations were functionally tested by transient gene expression in barley single epidermal leaf cells. Mutant variants that exhibited significant reduction in host cell entry compared to the respective wild-type *Mlo* are being combined through breeding. Currently, several combinations with simultaneous heterozygous mutants in the three *Mlo* homoeologs are being propagated. The resulting wheat triple homozygous mutant lines are expected to confer durable broad-spectrum powdery mildew resistance with reduced early senescence effects.

Sunflower-Pathogen Field Interaction: Spatial Patterns of the Disease Using Geostatistics Applied to Fixed Replicated Controls

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Effective disease evaluation relies on a better understanding on specific relationship between host and pathogen. This study presents a geostatistical approach to distinguish plant genetics and pathogen parameters in spatial pattern of Verticillium wilt Disease Severity (DS) of sunflower caused by the soil-borne fungus *Verticillium dahliae*.
Naturally infested sunflower fields were used to conduct the research. A randomized complete block design with 4 replicates was used. One susceptible (S) and one symptom-insensitive (SI) sunflower genotypes (controls) were introduced at fixed location within the field to establish a grid arrangement for the disease spatial pattern evaluation.
The S control shows a significant variation of DS indicating that the degree of heterogeneity was a function of environment. Geostatistical analyses were performed on both controls to evaluate part of micro-environment variability within the field that can interact with DS. Spatial structure analyses delivered the variogram function which was then used to calculate the degree of spatial dependence. Control values were interpolated to unsampled points through the ordinary kriging method. The degree of spatial autocorrelation differed between S and SI genotypes. Characterization of pathogen spatial variability was estimated by the S control deviation from the SI control associated value at all field coordinates. This infestation variation parameter was implemented in the general equation $P=G+E+G\times E+\varepsilon$ to introduce pathogen-centered data in the visual evaluation of susceptible/pathogen-insensitive genotypes. From this approach, disease evaluation can be refined taking out noise from infestation degree variation in final hybrid scores and can open a new path for *Verticillium dahliae* population pathogenicity studies.

Key words: Sunflower, *Verticillium dahliae*, Infestation Degree, Geostatistics, Disease Severity, Experimental Design

Session 8
Exploitation of novel resistance sources and strategies I

Chairs: Aska Goverse and Beat Keller

Recent developments in science and breeding to generate hybrid potato varieties with durable resistance to *Phytophthora infestans*.

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Potato is the third food crop in the world and provides excellent potentials to contribute to the future food security of the world as it has an efficient water usage and excellent nutrition quality, while the abundant genetic variation offers great potential for crop improvement. In addition, potato is one of the most studied crops and the complete genome and dozens of well-characterised resistant genes have been sequenced.

Since the Irish famine in the 1850's *Phytophthora infestans* has continuously threatened the cultivation of a healthy potato crop. Numerous resistance genes have been identified and sequenced. Most of these R-genes are NBS-LRR genes that recognize virulence proteins of the host. Resistance breeding for these genes have been inadequate due to the inefficient and slow traditional breeding process and the fast appearance of virulent isolates of the pathogen. Therefore, a cisgenic approach has been applied to combine different *R*-genes in commercial potato varieties in a more efficient process. This has been successful as cisgenic plants with combinations of *R*-genes remained resistant till the end of the growing season, while plants with single R-genes were not.

Alternatively, the F_1 hybrid breeding technology may be applied in potato, which has shown to be very effective to rapidly introduce new traits into hybrid varieties in other crops. This F_1 technology was believed not to be feasible in potato due to the self-incompatibility and strong inbreeding depression of diploid potato. Solynta has developed F_1 breeding technology in diploid potato by introducing the self-incompatibility gene (*Sli*) from *S. chacoense* and by a large scale breeding programme to eliminate the alleles with deleterious effects on plant growth and development.

Solynta will apply a marker assisted backcross strategy to rapidly introgress two different *R*-genes in the two parent lines of an experimental F_1 hybrid potato variety. This will deliver an essentially genetically identical F_1 hybrid with two resistance genes in a time period of only two years. Similarly, extra R-genes can be added. These may be specific *R*-genes as well as susceptibility derived *R*-genes. In this way the pathogen must overcome different defense barriers, which is expected to prove even more sustainable resistance.

Two examples where site-directed mutagenesis is attempted as a resistance breeding method; for aphid and net blotch resistance

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The view on how pests and diseases interact with their host plants has changed dramatically during the last century; from plants seen as passive sources of nutrition with preformed defences to highly dynamic relationships between the plant and its exploiters. So far, in breeding crops for resistance to pests and pathogens, the gene-for-gene type of resistance is most commonly exploited, with R-genes recognising the intruder and subseqent induction of plant defense genes. However, one example of successful plant breeding exploiting a different mechanism is resistance to powdery mildew in barley based on a natural mutation in the mildew resistance locus *mlo*. *MLO* encodes a protein that is a susceptibility factor, necessary for powdery mildew to successfully enter the epidermal host cells. Homozygous mutants in the *mlo*-locus are resistant to all current races of powdery mildew in barley and this resistance has been much used in European barley cultivars and still is. Here, two attempts to develop resistant breeding lines through mutations in other plant suceptibility genes are described, in these cases using recent site-directed mutagenesis techniques.

Aphids are severe pests in most of our crops and the long-term objective of the aphid project is to develop plants with durable resistance to aphids. This will be attempted by suppressing host susceptibility rather than introducing host resistance genes specific to each aphid species, and even aphid biotype, as has been the case in those few examples of successful breeding for aphid resistance in crop plants. First, we will identify host genes that aphids depend on, preferably those genes/gene types that are common to several aphid-host species combinations. After identifying candidate genes, via literature and database inventories, we will mutate selected genes in a site-specific way, in crops like wheat, barley, potato and pea.

The objective of the net blotch project is also to exploit host susceptibility (S) genes. However, in this pathosystem the necrotrophic fungal pathogen manipulates its host via necrotrophic effectors that specifically target host susceptibility targets, S-genes. This interaction which triggers host encoded programmed cell death pathways is typically utilized for resistance against biotrophic pathogens. We have access to two such genes in barley and

will attempt to mutate both in order to confirm their function in a first effort to introduce resistance to net blotch by suppressing necrotrophic effector triggered susceptibility (NETS).

There are now various molecular techniques available for editing particular DNA sites, with CRISPR/Cas9 and TALEN as the most recent. We have already applied TALEN technique in potato and are now also developing this as well as CRISPR/Cas9 for purposes described above.

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