PR-Proteins and Induced Resistance Against Pathogens and Insects

molecular biology meets application

Neuchâtel, Switzerland
4-8 September 2011

Abstract book



PR-proteins & Induced Resistance against pathogens & insects

PRIR 2011

September 4-8, Neuchâtel, Switzerland

The PRIR 2011 meeting is grateful for the support from the following sponsors and organisations:

IOBC-WPRS
OILB-SROP

unine
UNIVERSITÉ DE NEUCHÂTEL

PLANT SURVIVAL
National Centre of Competence in Research

sc | nat
Swiss Academy of Sciences
Akademie der Naturwissenschaften
Accademia di scienze naturali
Académie des sciences naturelles

Trends in Plant Science

syngenta

NOVARTIS

BASF
The Chemical Company

Merck Serono
Living science, transforming lives

Roche

Table of contents

I. General informations

Registration

Sunday 4 September: The registration desk will be open from 14:00 to 17:00. The registration desk is located in **room R.48**. For late arrivals, the desk will be open also on Monday morning. Upon arrival, the participants will receive a meeting welcome package including badge, USB stick, maps and touristic informations.

Meals

All lunches will be served in the restaurant le Romarin (③ on the site plan, 10 minutes walk from the congress site). Coffee breaks will be held at the cafeteria. Participants will receive further informations about restaurants included in the meeting welcome package. For any further needs, a nearby shopping mall („Maladière“, located at the football stadium) can be reached within 2 minutes of walk from the congress site.

Internet access

For the convenience of the participants, there is free internet access available at the congress site. Further information will be provided upon registration.

Talks and posters

All talks will be presented in the Aula des Jeunes Rives. Contributions should be submitted in an electronic format at least the day before the scheduled presentation. External laptops are not allowed to attach to the beamer; there will be a PC and Mac to transfer and pre-view the presentation. Due to the tense program, talks that do not respect the presentation time will be stopped according to the schedule.

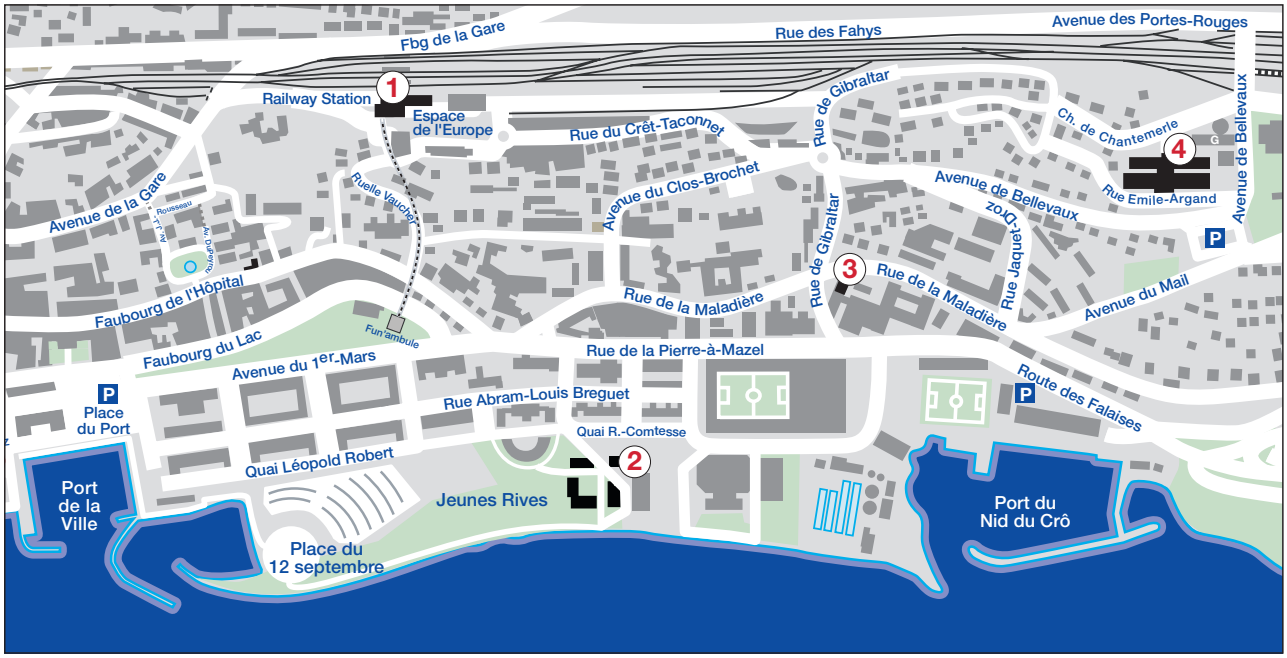
Posters with even numbers will be presented during the first poster session (Monday 5 September, 16:30 - 18:00), the second poster session (Tuesday 6 September, 17:30 - 19:00) is reserved for posters with odd numbers.

Meeting committee

For suggestions and comments concerning the PR IR 2011 meeting, feel free to conctact the PR IR 2011 organizing committee:

Brigitte Mauch-Mani (Brigitte.Mauch@unine.ch)
Corne M.J. Pieterse (C.M.J.Pieterse@uu.nl)
Annegret Schmitt, IOBC convenor (Annegret.Schmitt@jki.bund.de)

Site plan



1. Gare / Railway Station
2. Congress site : Aula des Jeunes-Rives
3. Restaurant le Romarin
4. UniMail

Sunday 4 September

14:00 - 17:00 **Registration** (the registration desk will be open on Monday for late arrivals)

17:00 - 19:00 **Welcome apéro** (at the congress site/registration site)

Monday 5 September

08:30 - 08:40 **Opening** (Brigitte Mauch-Mani, Corné Pieterse)

Session 1: Novel Players in plant defense signaling

Chair: Antonio Molina

08:40 - 09:20 Specificity of transcriptional responses to JA-Ile ***(Roberto Solano, SP)***

09:20 - 09:40 Transciptional activation and production of tryptophan-derived secondary metabolites in Arabidopsis roots contributes to the defense against the fungal vascular pathogen *Verticillium longisporum* **(*Wolfgang Dröge-Laser, GER)***

09:40 - 10:00 Transcriptome analysis of host and nonhost interactions between barley and the fungus *Magnaporthe* ***(Ulrich Schaffrath, GER)***

10:00 - 10:30 **Coffee break**

10:30 - 10:50 Adaptive evolution of the PRLIP gene family resulted in taxon-specific defense-related paralog groups in different plant species ***(Balint Szalontai, HU)***

10:50 - 11:10 Making sense out of signaling during plant defense ***(Corné Pieterse, NL)***

Session 2: Crosstalk between signaling pathways for biotic stress

Chair: Juriaan Ton

11:10 - 11:50 Cross-talk between defense signaling pathways for biotic stress ***(Christiane Gatz, GER)***

11:50 - 12:10 Rhizobacteria modify plant-aphid interactions: a case of induced systemic susceptibility ***(Ana Pineda, NL)***

12:10 - 14:00 **Lunch at Restaurant le Romarin**

14:00 - 14:20 Suppression of jasmonate signaling by salicylic acid acts downstream of SCFCOI1 and targets GCC-box promoter motifs in Arabidopsis **(*Saskia van Wees, NL)***

14:20 - 14:40 The role of the phytohormone ethylene in crosstalk between salicylate and jasmonate signaling ***(Antonio Leon-Reyes, EC)***

Session 3: The components of defense signaling 1

Chair: André Kessler

14:40 - 15:20 Interrelationships among SA, MeSA, lipids, and light in systemic acquired resistance (SAR) / The CRT1 family participates in multiple layers of resistance ***(Daniel F. Klessig, USA)***

15:20 - 15:40 GNOM ARF-GEF and ARF GTPase are linking multivesicular bodies to syntaxin-regulated penetration resistance ***(Hans Thordal-Christensen, DK)***

15:40 - 16:00 The Arabidopsis NIMIN cycle - shaping PR gene expression in the course of systemic acquired resistance ***(Ursula M. Pfitzner, GER)***

16:00 - 16:30 **Coffee break**

16:30 - 18:00 **Poster session I (posters with even numbers)** (with drinks)

evening Free time

Tuesday 6 September

Session 4: The components of defense signaling 2

Chair: Maria José Pozo

08:30 - 08:50 A permeable cuticle is associated with the release of reactive oxygen species and induction of innate immunity ***(Floriane L'Haridon, CH)***

08:50 - 09:10 Identification of grapevine pattern recognition receptors involved in PAMP-triggered immunity against *P. viticola* and *B. cinerea* ***(Lucie Trda, FR)***

09:10 - 09:30 SignWALLing: Signals derived from Arabidopsis cell wall activate specific resistance to pathogens ***(Antonio Molina, SP)***

09:30 - 09:50 Metabolic regulation of acquired resistance in Arabidopsis ***(Jürgen Zeier, GER)***

09:50 - 10:20 **Coffee break**

Session 5: Induced defense against insects

Chair: Christiane Gatz

10:20 - 11:00 The Arabidopsis-*Bemisia tabaci* interactions: Deciphering plant responses and insect behaviors in susceptible and resistant genotypes ***(Linda Walling, USA)***

11:00 - 11:20 Insect eggs suppress plant defence against herbivory in Arabidopsis ***(Philippe Reymond, CH)***

11:20 - 11:40 (Z)-3-Hexenol emitted from common cutworm-infested tomato plants induces jasmonate-independent defence to the intact tomato plants against upcoming herbivore attack ***(Koichi Sugimoto, JP)***

11:40 - 12:00 A root herbivore induces susceptibility to conspecifics in maize ***(Christelle Robert, CH)***

12:00 - 13:40 **Lunch at Restaurant le Romarin**

Session 6: Induced defense against pathogens - practical aspects

Chair: Xavier Daire

13:40 - 14:00 Effect of nitrogen fertilisation of strawberry plants on the efficacy of defence-stimulating biocontrol products against *Botrytis cinerea* ***(Philippe C. Nicot, FR***)

14:00 - 14:20 Time effectiveness using of inducers of chemical and biological origin against powdery mildew (*Blumeria graminis* f. sp. *tritici*) on wheat ***(Lubomir Vechet, CZ)***

14:20 - 14:40 What tools used to acquire a better understanding of induced resistance after elicitation and\or infection from laboratory to field experiments? ***(Marie-France Corio - Costet, FR)***

14:40 - 15:00 Diversity in susceptibility of *Botrytis cinerea* to biocontrol products inducing plant defence mechanisms ***(Marc Bardin, FR)***

15:00 - 15:30 **Coffee break**

Session 7: Induced defense against pathogens

Chair: Jürgen Zeier

15:30 - 16:10 Abietane diterpenoid in plant defense signaling! ***(Jyoti Shah, USA)***

16:10 - 16:30 Differential effects of biotic and abiotic elicitors on plant defence and physiology in radiata pine seedlings ***(Tony Reglinski, NZ)***

16:30 - 16:50 Specific phosphorylation of conserved eukaryotic protein SGT1 is an essential element of plant defense against pathogens ***(Magdalena Krzymowska, PL)***

16:50 - 17:10 The hemibiotrophic fungus *Colletotrichum graminicola* triggers above and belowground systemic resistance in *Zea mays* ***(Dirk Balmer, CH)***

17:10 - 17:30 Characterization of *Trichoderma harzianum* T39-induced resistance of grapevine against downy mildew ***(Michele Perazzolli, IT)***

17:30 - 19:00 **Poster session II (posters with odd numbers)** (with drinks)

19:00 Free time

Wednesday 7 September

Session 8: Molecular ecology of induced defense against insects

Chair: Philippe Reymond

08:30 - 09:10 Information war in the plant headspace ***(Andre Kessler, USA)***

09:10 - 09:30 Multi-species multitrophic plant-insect interactions: from ecology to genes ***(Roxina Soler, NL)***

09:30 - 09:50 Symbiotic ants as an indirect defence against phytopathogens in an ant-plant mutualism ***(Marcia González-Teuber, GER)***

09:50 - 10:20 **Coffee break**

10:20 - 10:40 Different systemic induced defense responses in roots and shoots after an above- or belowground jasmonic acid application ***(Tom Tytgat, NL)***

10:40 - 11:00 MYB8 transcription factor: a universal regulator of inducible phenolamides in *Nicotiana attenuata* after herbivory ***(Nawaporn Onkokesung, GER)***

Session 9: Induced defense by beneficials

Chair: Bruno Cammue

11:00 - 11:40 Unravelling mycorrhiza-induced resistance ***(Maria José Pozo, SP)***

11:40 - 12:00 *Trichoderma*-induced plant responses to abiotic and biotic stimuli ***(Ada Viterbo, IL)***

12:00 - 13:50 **Lunch at Restaurant le Romarin**

13:50 - 14:30 Jasmonates in biotic interactions of roots ***(Bettina Hause, GER)***

14:30 - 14:50 The bacterial N-acyl homoserine lactone oxo-C14-HSL from the tripartite *Piriformospora* symbiosis confers disease resistance via activation of AtMPK6 and AtMPK3 ***(Karl-Heinz Kogel, GER)***

14:50 - 15:10 AM symbiosis as a control strategy against root parasitic plants through strigolactone reduction ***(Juan A. López-Ráez, SP)***

15:10 - 15:40 **Coffee break**

Session 10: Priming for resistance

Chair: Karl-Heinz Kogel

15:40 - 16:20 How innate is the plant immune system?! ***(Jurriaan Ton, UK)***

16:20 - 16:40 The genetically enriched (E)-β-ocimene in transgenic plants functions in indirect defenses in the both transgenic plants themselves and neighboring plants ***(Gen-ichiro Arimura, JP)***

16:40 - 17:00 The role of root-specific MYB72 transcription factor during rhizobacteria-induced systemic resistance in Arabidopsis ***(Christos Zamioudis, NL)***

17:00 - 17:20 Lectin receptor kinases as modulators of the Arabidopsis innate immunity response ***(Laurent Zimmerli, TW)***

17:20 - 17:40 Molecular aspects of defense priming ***(Uwe Conrath, GER)***

19:30 - ??? **Social dinner (Restaurant Le Romarin) - announcing of poster prizes**

Thursday 8 September

Session 11: The future of induced resistance

Chair: Corné Pieterse

09:00 - 09:40 Properties and structure of the plant immune signaling network ***(Fumiyaki Katagiri, USA)***

09:40 - 10:00 Next generation Systemic Acquired Resistance ***(Estrella Luna-Diez, UK)***

10:00 - 10:20 Discovery of WRKY Transcription Factors Involved in Activation of SA Biosynthesis Genes: a Bioinformatics and Molecular Biology Approach ***(Marcel Van Verk, NL)***

10:20 - 10:50 **Coffee Break**

10:50 - 11:10 New chromatographic approaches reveal an active role of glucosyl salicylates against *P. syringae* in priming mutants ***(Victor Flors, SP)***

11:10 - 11:40 Closing lecture ***(Brigitte Mauch-Mani, CH)***

11:40 - 12:00 Closing remarks

12:00 Departure

Specificity of transcriptional responses to JA-Ile

Presenting author: **Roberto Solano** (rsolano@cnb.csic.es)

Dept. Genética Molecular de Plantas, Centro Nacional de Biotecnologia-CSIC, Universidad Autonóma de Madrid, Spain

Jasmonate-Isoleucine (JA-Ile) triggers an important transcriptional reprogramming of plant cells to modulate both basal development and stress responses. The core JA signaling module has been recently molecularly defined as the SCF^{COI1}-JAZ-MYC2 complex. Within this module, COI1 and JAZ proteins act as JA-Ile co-receptors regulating the activity of the TF MYC2 in response to the hormone. In spite of the importance of transcriptional regulation, only one transcription factor (TF), the bHLH MYC2, has been described so far as a direct target of JAZ repressors. However, MYC2 can not explain the diversity of plant responses regulated by JA-Ile. It has been speculated that the specific interactions between JAZs and their respective (still unidentified) TF targets may be largely responsible for the diversity and specificity of JA-Ile responses to different stimuli.

We have recently identified two additional bHLH TFs, MYC3 and MYC4 and showed that they are direct targets of JAZ repressors, acting additively with MYC2 in the activation of JA responses. Both TFs are required for full responsiveness to the hormone in several JA-regulated physiological processes, including gene expression, inhibition of root growth, and pathogen and insect resistance. Interestingly, although the genetic analysis demonstrated some specificity in the function of MYC2, MYC3 and MYC4, biochemical and molecular results were consistent with the three proteins being functionally redundant. Thus, we hypothesize that rather than differences in JAZ-TF interactions the diversity of JA-Ile responses in the plant may be specified by the differences in tissue expression patterns of mostly redundant TFs.

Transciptional activation and production of tryptophan-derived secondary metabolites in *Arabidopsis* roots contributes to the defense against the fungal vascular pathogen *Verticillium longisporum*

Presenting author: **Wolfgang Dröge-Laser** (wolfgang.droege-laser@uni-wuerzburg.de)

Authors: Iven T[1], Götze S[1], Singh SA[2], Braus-Stromeyer S[2], Lipka V[1], Feussner I[1], Dröge-Laser W.[1,3]

[1]Albrecht-von-Haller Institut fuer Pflanzenwissenschaften, Georg-August-Universitaet Goettingen, Untere Karspuele 2, 37073 Goettingen, Germany

[2] Institut ür Mikrobiologie und Genetik, Georg-August-Universitaet Goettingen, Grisebachstr. 8, 37077, Germany3

[3]Julius-von-Sachs-Institut, Pharmazeutische Biologie, Julius-Maximilians-Universitaet Wuerzburg, Julius-von-Sachs-Platz 2, 97082 Wuerzburg, Germany

Plant defence efficacy results of interactions of various factors, including physiological and genetic characteristics of both partners in host-pathogen interaction as well as environmental conditions. Numerous studies showed an excellent efficacy of elicitors in laboratory conditions, but *in natura* the obtained results are often disappointing.

To acquire a better understanding of grapevine defence status in vineyard, after elicitor applications with benzothiadiazole or phosphonates, we have developed a triple approach (biological, biochemical and molecular) in our laboratory.

From experiments performed in laboratory, we choose a set of selected genes potentially involved in grapevine defense. After an assessment to estimate their interest as markers of grapevine defenses by RT-qPCR at different times, we have also assessed the impact of pathogen variability intra or inter species on the efficacy of elicitors. Finally, transcripts were specifically up or down regulated in infected-leaves according to elicitors or the intra and inter specific variability of pathogen. To complete the transcript approach, quantitative analysis of polyphenols and biological efficacy were also performed to correlate the different approaches to the efficacy of grapevine defenses. So we have expression patterns and polyphenol profiles of infected-leaves that could be use as potential markers to vineyard experiments.

In 2009 and 2010, these markers have been tested to estimate the defense level of molecules as benzothiadiazole, a phosphonate, and mancozeb (fungicide reference), after *Plasmopara viticola* artificial inoculation in vineyard. The samplings, the analyses, the epidemiological follow-up and the quality of the grape harvested showed that the grapevine defenses were correlated well at the level of protection and that some compounds have an effect on the development which seem directly connected to climatic and vintage year. Surprisingly the better up-regulation of transcripts was obtained with the fungicide mancozeb.

To date we thus have tools allowing to understand how reacts the plant in its agronomic environment, which should allow us to exploit and to set up better the use of elicitors in alternative or complementary strategies of grapevine pest management.

Transcriptome analysis of host and nonhost interactions between barley and the fungus *Magnaporthe*

Presenting author: **Ulrich Schaffrath** (schaffrath@bio3.rwth-aachen.de)

Authors: Delventhal R, Mogga V, Weidenbach D, Schaffrath U

Department of Plant Physiology, RWTH Aachen University, D-52056, Aachen, Germany

Members of the fungal genus *Magnaporthe* cause blast disease on over 50 grass species including the economically important cereals rice, wheat, barley and millet. Barley establishes a host-type of interaction with *M. oryzae* and a nonhost-type of interaction with *Magnaporthe* species isolated from the grass genera *Digitaria* or *Pennisetum*. We aimed at identifying the genetic framework of the latter nonhost scenario in a holistic approach considering both the fungal and plant interaction partner.

On the plant side, we performed transcription profiling of peeled barley epidermis infected with *Magnaporthe* host or nonhost isolates. Expression profiles of barley genes that are differentially regulated in the host versus nonhost interaction were identified. Functional analysis of these candidate genes was done by using barley stripe mosaic virus-induced gene silencing. This work contributes to the ERA-PG network 'TritNonhost' which encompasses the integrative genomic and genetic analysis of nonhost resistance in wheat and barley to rust (*Puccinia* spp.), powdery mildew (*Blumeria* spp.) and blast (*Magnaporthe* spp.).

On the fungal side, we investigated whether secreted fungal molecules which suppress plant defences might determine the infection success of *Magnaporthe* host isolates on barley. In agreement with this hypothesis, a simultaneous inoculation of barley with an adapted and non-adapted *Magnaporthe* isolate improved the ability of the ostensible nonhost to overcome defence barriers. To identify potentially involved effector genes a comparative transcriptome analysis of *Magnaporthe* host and nonhost isolates during their interaction with barley was conducted. Expression profiles of different candidate genes (*HEG*, hypothetical effector gene) were generated using RT-qPCR. Interestingly, the maximum expression level of each *HEG* corresponds to distinct developmental stages of the fungus. Functional analysis of *HEG*s either by overexpression studies or by gene knock-out is in progress.

Adaptive evolution of the *PRLIP* gene family resulted taxon-specific defense-related paralog groups in different plant species

Presenting author: **Balint Szalontai** (balint@gamma.ttk.pte.hu)

Authors: Szalontai B[1] and Jakab G[1,2]

[1]University of Pecs, Faculty of Sciences, Institute of Biology

[2]University of Pecs, Faculty of Sciences, Institute of Viticulture and Oenology

PRLIP (pathogenesis-related lipase) is a gene family identified in *Arabidopsis thaliana* with 9 members encoding class 3 lipase-like proteins. Members of the family exhibited typical expression characteristics of genes encoding pathogenesis-related (*PR*) proteins. *PRLIP1, PRLIP2* and *PRLIP6* paralogs displayed enhanced expression during pathogen infection and in respond to different stress hormone treatments. Besides these inducible genes, other members of the gene family - *PRLIP3, PRLIP8* and *PRLIP9* - showed relatively high basic transcript levels in Arabidopsis leaves and were hardly stimulated by biotic stress. Salinity stress caused by NaCl disposal, however, increased both *PRLIP3* and *PRLIP9* transcriptions. All of the expressed *PRLIP* genes showed an organ specific expression pattern but again the lower differences among the tested tissues were found in cases of *PRLIP3/9* and *PRLIP8*. An extensive screening of the public plant genome databases revealed that orthologs of the latter three genes are commonly found in all sequenced plant genomes, thus we named them core *PRLIP*s. Besides these core genes, in several (but not every) sequenced plant species taxon-specific *PRLIP* groups have been identified, which are likely to be evolved independently, mostly via tandem gene duplication. Apparently, the formerly characterized *PRLIP1, PRLIP2* and *PRLIP6* genes together with *PRLIP4*, *PRLIP5* and *PRLIP7* form such a taxon-specific *PRLIP* group of *A. thaliana*. We hypothesized, that these unique clusters of *PRLIP*s might have been evolved to attend plant pathogen responses and protection, while core *PRLIP*s possibly serve other functions. To test this idea we examined the *PRLIP* gene family of grapevine (*Vitis vinifera*) consisting of two core *PRLIP* genes (*VvPRLIP3* and *VvPRLIP8*) and two taxon-specific members termed *VvPRLIPA* and *VvPRLIPC*. In case of both grapevine specific *VvPRLIP*s, transcription was highly induced by powdery mildew (*Erysiphe necator*) infection and also by treatment with the SAR-inducing chemical benzothiadiazole (BTH). Conversely, ethylene treatment reduced their expression levels to almost undetectable. However, both powdery mildew infection and chemical treatments had only minor influence on mRNA levels of the *VvPRLIP3* and *VvPRLIP8* genes. Similarly, no relevant organ specific expression differences among the grape core *PRLIP* genes could be detected. These findings further supported our hypothesis that core *PRLIP* genes are rather “PR-like” genes (PRLs – according to van Loon’s original nomenclature) in contrast to the taxon-specific members of the gene family showing veritable PR characteristics with regard to vigorous induction by pathogen attack and organ specific differences in basal gene expression as we have observed in both Arabidopsis and grapevine. Our observation that - similarly to PR1 gene family - the defense-related members of PRLIP gene family evolved to taxon-specific paralog groups indicates a common scheme in the adaptive evolution of PR genes.

Making sense out of signaling during plant defense

Presenting author: **Corné M.J. Pieterse** (C.M.J.Pieterse@uu.nl)

Authors: Pieterse CMJ, Leon-Reyes A, Van der Does D, Verhage A, Zamioudis C, Pel C, Coolen S, Vos I, Van Verk MC, Van Pelt JA, Van Wees SCM

Plant-Microbe Interactions, Faculty of Science, Utrecht University, The Netherlands

Plants live in complex environments in which they intimately interact with a broad range of other organisms. Signaling networks that are recruited in response to parasitic and beneficial organisms can overlap, indicating that the regulation of the plant's adaptive response to its biotic environment is finely balanced between protection against aggressors and acquisition of benefits[1-3]. Hormones such as salicylic acid, jasmonic acid (JA) and ethylene play pivotal roles in the regulation of the defense signaling network[4]. Their signaling pathways interact, providing the plant with a powerful capacity to tailor its immune response to the attacker encountered. As plants co-evolved with an enormous variety of alien organisms, they harbour a fantastic reservoir of natural adaptive mechanisms. We study natural plant defense signaling networks and focus on the question: how are plants capable of integrating microbial- and insect-induced signals into defenses that are specifically directed against the attacker? By placing molecular mechanisms into an evolutionary and ecological context we try to make sense out of signaling during plant defense.

An example of our research comes from the observation that plant defenses against insect herbivores and necrotrophic pathogens are differentially regulated by different branches of the JA signaling pathway, which are antagonistically regulated by MYC2 and ERF-type transcription factors. Mutant analysis revealed that feeding by larvae of the specialist insect herbivore *Pieris rapae* activated the MYC2-branch of the JA pathway, while it suppressed the ERF-branch., indicating that the MYC2-branch is prioritized over the ERF-branch during insect feeding. In a two-choice setup the larvae consistently preferred genotypes in which the ERF-branch was activated over genotypes in which the MYC2-branch was induced, suggesting that the herbivores were stimulated to feed from plants that expressed the ERF-branch rather than that they were deterred by plants that expressed the MYC2-branch. Interestingly, application of larval oral secretion into wounded leaf tissue stimulated the ERF-branch of the JA pathway, suggesting that compounds in the oral secretion have the potential to manipulate the plant response toward the caterpillar-preferred ERF-regulated branch of the JA response. Our results suggest that by activating the MYC2-branch of the JA pathway, plants prevent stimulation of the ERF-branch by the herbivore, thereby becoming less attractive to the attacker.

1. Pieterse CMJ, Dicke M. Plant interactions with microbes and insects: from molecular mechanisms to ecology. Trends Plant Sci. 12, 564-569 (2007).
2. Van Wees SCM, Van der Ent S, Pieterse CMJ. Plant immune responses triggered by beneficial microbes. Curr. Opin. Plant Biol. 11, 443-448 (2008).
3. Van der Ent S, Van Wees SCM, Pieterse CMJ. Jasmonate signaling in plant interactions with resistance-inducing beneficial microbes. Phytochemistry 70, 1581-1588 (2009).
4. Pieterse CMJ, Leon-Reyes A, Van der Ent S, Van Wees SCM. Networking by small-molecule hormones in plant immunity. Nature Chem. Biol. 5, 308-316 (2009).

Cross-talk between defense signaling pathways for biotic stress

Presenting author: **Christiane Gatz** (cgatz@gwdg.de)

Authors: Gatz C, Zander M

Albrecht von Haller Insitute for Plant Sciences, Georg-August-University Göttingen, Untere Karspüle 2, 37073 Göttingen

Plants are constantly exposed to different pathogens to which they react with a battery of induced defense mechanisms. The corresponding signal transduction pathways are highly interconnected so that cellular responses can be modulated depending on the combination of attackers with different life styles. Here we show that bZIP transcription factors of the TGA family, which are involved in mediating gene expression within the salicylic acid pathways, directly activate the promoter of the *ORA59* gene which encodes the global regulator of the jasmonic acid (JA)/ethylene (ET)-induced defense program. In the *tga256* mutant, which lacks the closely related class II TGA factors TGA2, TGA5 and TGA6, ET-, but not JA-induced transcription of the *ORA59* promoter is compromised. Chromatin immunoprecipitation experiments and analyses of transgenic lines carrying different $ORA59_{Pro}$*:GUS* fusions collectively suggest that class II TGA factors are recruited to the TGACG binding site of the *ORA59* promoter under conditions of enhanced ET levels. Microarray analysis of wildtype and *tga256* mutant plants treated with the ET precursor ACC yielded 193 ACC-regulated genes that did not respond to ACC in the *tga256* mutant. To challenge the hypothesis whether the strong negative effect of SA on ET-induced genes involves TGA factors, we compared the transcriptomes of wild-type and *tga256* mutants after the combined treatment with ACC and SA. This documented that all the ACC-induced genes that are suppressed by SA require TGA proteins for activation. The negative effects of SA and the JA-responsive transcription factor MYC2 are mediated by SA- and MYC2-induced glutaredoxins which physically interact with TGA factors. Unlike wild-type plants, *tga256* mutant plants did not react with a SA-induced increase in susceptibility towards *Botrytis cinerea* underpinning the notion that TGA factors provide the molecular link between SA and the plant defense program against necrotrophic pathogens.

Rhizobacteria modify plant-aphid interactions: a case of induced systemic susceptibility

Presenting author: **Ana Pineda** (ana.pineda@wur.nl)

Authors: Pineda A, Zheng S-J, van Loon JJA, Dicke M

Laboratory of Entomology, Wageningen University

Several beneficial microbes such as plant growth-promoting rhizobacteria and mycorrhizal fungi have a plant-mediated effect on insects aboveground. The plant growth-promoting rhizobacterium *Pseudomonas fluorescens* can induce systemic resistance in *Arabidopsis thaliana* against several microbial pathogens and chewing insects. However, the plant-mediated effect of these beneficial microbes on phloem feeding insects is not well understood.

Using *Arabidopsis* as a model, we here report that *P. fluorescens* has a positive effect on the performance (weight gain and intrinsic rate of increase) of the generalist aphid *Myzus persicae* but no effect on the crucifer specialist *Brevicoryne brassicae*. Additionally, transcriptional analyses of selected genes revealed that in the plant-microbe interaction with *M. persicae*, rhizobacteria a) prime the plant for an enhanced expression of *LOX2*, a gene involved in the jasmonic acid (JA)-defense pathway, and b) suppress the expression of *ABA1*, a gene involved in the abscisic acid (ABA) signaling pathway, at several time points. In contrast, no effect in the plant-microbe interaction with *B. brassicae* was found at the transcriptional level.

This study presents the first data on rhizobacteria-induced systemic susceptibility to an herbivorous insect, supporting the pattern proposed for other beneficial microbes belowground and phloem feeders aboveground. Moreover, we provide further evidence that at the transcript level, soil-borne microbes modify plant-aphid interactions.

Suppression of jasmonate signaling by salicylic acid acts downstream of SCF^{COI1} and targets GCC-box promoter motifs in *Arabidopsis*

Presenting author: **Saskia Van Wees** (s.vanwees@uu.nl)

Authors: Van der Does D, Leon-Reyes A, Koornneef A, Rodenburg N, Memelink J, Pieterse CMJ, Van Wees SCM

Plant-Microbe Interactions, Utrecht University, P.O. Box 800.56, 3508 TB Utrecht, The Netherlands.

The signaling molecules salicylic acid (SA) and jasmonic acid (JA) play major roles in the plant immune signaling network. The SA- and JA-controlled signaling pathways can cross-communicate leading to a finely tuned plant defense response. In *Arabidopsis thaliana,* SA suppresses expression of JA-responsive genes, among which *PDF1.2* and *VSP2*. We aim to unravel how SA exerts its antagonistic effect on JA-responsive gene expression and at which level in the JA signaling pathway SA is acting. CORONATINE INSENSITIVE1 (COI1), which is part of the SCF^{COI1} complex, is an essential component of the JA signaling pathway. By overexpressing the transcription factor ERF1, expression of *PDF1.2* is rescued in the *coi1-1* mutant background (Lorenzo et al., 2003). Here we show that SA can still suppress ERF1-induced *PDF1.2* expression in *35S::ERF1/coi1-1* plants, demonstrating that SA can target the JA signaling pathway downstream of SCF^{COI1} and of ERF1. SA was shown not to affect ERF1 protein accumulation, indicating that the antagonistic effect of SA on ERF1-mediated gene expression was not a result of reduced ERF1 accumulation. ERF1 binds to GCC-box promoter motifs; genome-wide promoter analysis of JA-inducible genes that are suppressed by SA revealed an overrepresentation of the GCC-box. Using plants carrying the *GUS* reporter gene under control of the GCC-box, we demonstrated that the GCC-box is a sufficient element for SA-induced suppression of JA-induced gene expression. We speculate that SA might repress JA signaling via interference with binding of JA-dependent ERF transcription factors to the GCC-box.

The role of the phytohormone ethylene in crosstalk between salicylate and jasmonate signaling

Presenting author: **Antonio Leon-Reyes** (aleon@usfq.edu.ec)
Authors: Leon-Reyes A[1], Spoel SH[1], De Lange ES[2], Pieterse CMJ[2]
[1]Universidad San Francisco de Quito, Colegio de Agricultura, Alimentos y Nutrición
[2]Plant-Microbe Interactions, Department of Biology, Faculty of Science, Utrecht University

The plant hormones salicylic acid (SA), jasmonic acid (JA), and ethylene (ET) play crucial roles in the signaling network that regulates induced defense responses against biotic stresses. Antagonism between SA and JA operates as a mechanism to fine-tune defenses that are activated in response to multiple attackers. In Arabidopsis (*Arabidopsis thaliana*), NONEXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1) was demonstrated to be required for SA-mediated suppression of JA-dependent defenses. Since ET is known to be an important modulator of defense responses, we investigated the role of ET in the SA-JA signal interaction. We found two modulating roles of ET. First, pharmacological and biological experiments with gaseous ET and the ET precursor 1-aminocyclopropane-1-carboxylic acid showed that ET potentiated SA/NPR1-dependent *PATHOGENESIS-RELATED1* transcription, while it rendered the antagonistic effect of SA on methyl jasmonate-induced *PDF1.2* and *VSP2* expression NPR1 independent. Our results suggest a model in which ET modulates the NPR1 dependency of SA-JA antagonism, possibly to compensate for enhanced allocation of NPR1 to function in SA-dependent activation of *PR* genes. Second, a mutant *cev1*, which displays constitutive expression of JA and ET responses, appeared to be insensitive to SA-mediated suppression of the JA-responsive marker genes *PDF1.2* and *VSP2*. Pharmacological assays, mutant analysis, and studies with the ET signaling inhibitor 1-methylcyclopropene revealed that ET signaling renders the JA response insensitive to subsequent suppression by SA. Collectively, our results point to a model in which ethylene plays a fundamental role in cross-talk between SA and JA, thus modulating the final plant response during multiple attacker interactions.

Interrelationships among SA, MeSA, lipids, and light in systemic acquired resistance (SAR) / The CRT1 family participates in multiple layers of resistance

Presenting author: **Daniel F. Klessig** (dfk8@cornell.edu)

Authors: Klessig DF[1], Kang HG[1], Liu PP[1], von Dahl C[1], Choi HW[1], Park SW[1], Kogel KH[1], and Kachroo P[2]

[1]Boyce Thompson Institute for Plant Research, Ithaca, NY, USA
[1]Dept. Plant Pathology, University of Kentucky, Lexington, KY, USA

SAR is a state of heightened defense induced throughout a plant following local infection by a pathogen. Development of SAR involves synthesis of a mobile signal(s) at the site of infection and its translocation through the vascular system to distal (systemic) tissues. Results from our group argue that methyl salicylate (MeSA) serves as this mobile SAR signal in tobacco, Arabidopsis, and potato (Park et al., Science, 2007; Vlot et al., Plant Journal, 2008; Park et al., JBC, 2009; Liu et al., MPMI, 2010; Manosalva et al., MPMI, 2010). In contrast, Zeier and coworkers presented results which suggest that MeSA is not essential for SAR (Attaran et al., Plant Cell, 2009). We have identified the difference in experimental design which accounts for these conflicting results. Under certain light conditions MeSA is required for SAR signaling while under other light conditions it plays only an auxiliary role.

In addition to MeSA, one or more lipid-based mobile signals have been implicated in systemic immunity by several groups. Our analyses of mutants in the lipid-transfer protein DIR1 and of plants over expressing *BA/SAMT1* suggests that under certain conditions SAR is activated via the interplay between at least two mobile signals, MeSA and a complex formed between DIR1 and a lipid or lipid derivative. The function of this complex is to suppress expression and/or activity of BA/SAMT1 in the distal tissue to facilitate conversion by MeSA esterases of the translocated MeSA to biologically active SA for induction and potentiation of defense responses (Liu et al., Plant Physiol. 2011).

A genetic screen in Arabidopsis for mutants **C**ompromised for **R**ecognition of **T**urnip Crinkle Virus *via* the HRT R (resistance) protein identified CRT1. CRT1 is an endosomal-localized novel ATPase, which interacts with 11 R proteins from different structural classes. This interaction is dynamic since R protein activation appears to disrupt it. This result, together with CRT1's function upstream of Ca^{2+} fluxes and MAP kinase activation, argue that CRT1 plays a critical role early in *R* gene-mediated defense signaling. The Arabidopsis mutant *crt1-2 crh1-1,* which lacks CRT1 and its closest homolog, displayed compromised resistance to avirulent *P. syringae* and *Hyaloperonospora arabidopsidis* (Kang et al., Cell Host Microbe 2008 and Plant Cell 2010). Recent analyses of *crt1-2 crh1-1* revealed that CRT1 is also required for basal resistance against TCV and *P. syringae*, for non-host resistance to *Phytophthora infestans*, and for SAR. Moreover, it interacts with the PAMP recognition receptors FLS2 and EFR and with their associated kinases BAK1 and BIK1. Together, these results suggest that CRT1 is a crucial player in four levels of plant immunity against a wide range of pathogens. Potential mechanisms by which the CRT1 family modulates plant immunity will be discussed.

GNOM ARF-GEF and ARF GTPase are linking multivesicular bodies to syntaxin-regulated penetration resistance

Presenting author: **Hans Thordal-Christensen** (htc@life.ku.dk)

Authors: Thordal-Christensen H, Nielsen ME, Böhlenius H

Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Denmark

Basal defence against powdery mildew fungi is manifested as penetration resistance in the outer cell wall of epidermal cells. This involves formation of a callose-containing cell wall apposition, called a papilla, at the site of fungal penetration. The orthologous plasma membrane syntaxins, PEN1 and ROR2, of Arabidopsis and barley have previously been implicated in penetration resistance. These syntaxins accumulate at the site of attack as they become embedded in the papilla, and we consider these as markers for exosomes secreted during the build-up of the cell wall apposition. Syntaxins belong to the SNARE proteins involved in vesicle fusion. Meanwhile, vesicle budding is regulated by ARF GTPases, which in turn are activated by ARF guanine nucleotide exchange factors (ARF-GEFs).

We found that BFA, that targets certain ARF-GEFs, inhibits penetration resistance in Arabidopsis. Furthermore, BFA inhibits deposition of callose and GFP-PEN1-labelled exosomes in papillae. By introducing a BFA-resistant version of *At*GNOM, we were able to make these depositions BFA-insensitive. This and other studies demonstrated that *At*GNOM is involved in penetration resistance.

In a parallel study in barley, we used transient single cell RNAi-based gene silencing to screen for ARF GTPases involved in penetration resistance. Thereby, we identified *Hv*ARFA1b/1c to be essential for this type of basal defence. Subsequent analyses using over-expression of dominant-negative versions of this *Hv*ARFA1b/1c demonstrated that it is important for ROR2-regulated penetration resistance and deposition of callose and YFP-ROR2 in papillae. Confocal studies of *Hv*ARFA1b/1c-GFP associated it with multivesicular bodies.

The Arabidopsis NIMIN cycle – shaping PR gene expression in the course of systemic acquired resistance

Presenting author: **Ursula M. Pfitzner** (pfitzner@uni-hohenheim.de)

Authors: Maier F, Hermann M, Pfitzner UM

Universität Hohenheim, Institut für Genetik, FG Allgemeine Virologie, D-70593 Stuttgart

Systemic acquired resistance (SAR) is a long lasting and broad-spectrum disease resistance state in plants which is elicited by expression of the hypersensitive response (HR). Hallmarks of SAR are the signal molecule salicylic acid (SA) and the induction of *PATHOGENESIS-RELATED (PR) PROTEIN-1* genes. The SA signal is transduced through the regulatory protein NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1; Cao et al., 1997; Ryals et al., 1997). NPR1 interacts with two groups of proteins, TGA transcription factors and NIM1-INTERACTING (NIMIN) proteins (Zhang et al., 1999; Weigel et al., 2001). TGA factors link NPR1 to SA responsive *cis*-acting elements present in the promoter regions of *PR-1* genes (Strompen et al., 1998), while NIMIN proteins regulate NPR1 activity (Weigel et al., 2005; Chern et al., 2005; Zwicker et al., 2007). There are four *NIMIN* genes in Arabidopsis, *NIMIN1*, *NIMIN1b*, *NIMIN2* and *NIMIN3*. Recently, Arabidopsis *NIMIN* genes have been classified as lineage-specific genes (LSGs; Donoghue et al., 2011). The group of LSGs encompasses fast evolving genes that are important for environmental adaptation of the plant response to various stress conditions. Consistently, biochemical differences have been recognized between NIMIN and NPR1 proteins from Arabidopsis, tobacco and rice (Zwicker et al., 2007; Maier et al., 2011; Chern et al., 2005). Recent evidence suggests that SA targets NPR1 directly. The SA signal is transmitted through two conserved domains in the C-terminal third of NPR1 (Maier et al., 2011; Canet et al., 2010). One domain comprises the penta-amino acid motif LENRV. The other domain comprises the binding site for the SA-induced NIMIN1 and NIMIN2-type proteins. NPR1-related proteins that contain these two conserved C-terminal domains are likewise sensitive to SA. To further unravel the functional significance of NIMIN binding for signal transduction through NPR1, we have studied the expression of the Arabidopsis *NIMIN* genes, the interaction of the encoded proteins with the NPR1/TGA complex, and the impact of NIMIN proteins on the activity of NPR1. Together, our results show that the Arabidopsis NIMIN proteins act on NPR1 at different times and in different modes suggesting that they supervise the central SAR inducer NPR1 to promote *PR* gene expression at distinct stages of SAR.

Canet et al. (2010) Plant, Cell & Environ. 33, 1911-1922.
Cao et al. (1997) Cell 88, 57-63.
Chern et al. (2005) Plant J. 43, 623-635.
Donoghue et al. (2011) BMC Evolutionary Biology 11:47.
Maier et al. (2011) Mol. Plant Pathol. 12, 73-91.
Ryals et al. (1997) Plant Cell 9, 425-439.
Strompen et al. (1998) Plant Mol. Biol. 37, 871-883.
Weigel et al. (2001) Plant Mol. Biol. 46, 143-160.
Weigel et al. (2005) Plant Cell 17, 1279-1291.
Zhang et al. (1999) Proc. Natl. Acad. Sci. USA 96, 6523-6528.
Zwicker et al. (2007) Mol. Plant Pathol. 8, 385-400.

A permeable cuticle is associated with the release of reactive oxygen species and induction of innate immunity

Presenting author: **Floriane L'Haridon** (floriane.lharidon@unifr.ch)
Authors: L'Haridon F, Binda M, Serrano M, Abou-Mansour E, Métraux JP
University of Fribourg, Department of Plant Biology, Switzerland

Wounded leaves of *Arabidopsis thaliana* show transient immunity to *Botrytis cinerea*, the causal agent of grey mould. Using a fluorescent probe, histological staining and a luminol assay, we now show that reactive oxygen species (ROS), including H_2O_2 and O_2^- are produced within minutes after wounding. ROS are formed in the absence of the enzymes Atrboh D and F and can be prevented by diphenylene iodonium (DPI) or catalase. H_2O_2 was shown to protect plants upon exogenous application. ROS accumulation and resistance to *B. cinerea* were abolished when wounded leaves were incubated under dry conditions, an effect that was found to depend on abscisic acid (ABA). Accordingly, ABA biosynthesis mutants (*aba2* and *aba3*) were still fully resistant under dry conditions even without wounding. Under dry conditions, wounded plants displayed enhanced expression of ABA-dependent and ABA-reporter genes. Mutants impaired in cutin synthesis such as *bdg* and *lacs2.3* are already known to display a high level of resistance to *B. cinerea* and were found to produce ROS even when leaves were not wounded. An increased permeability of the cuticle and enhanced ROS production were detected in *aba2* and *aba3* mutants as described for *bdg* and *lacs2.3*. Moreover, leaf surfaces treated with cutinase produced ROS and became more protected to *B. cinerea.* Thus, increased permeability of the cuticle is strongly linked with ROS formation and resistance to *B. cinerea*. The amount of oxalic acid, an inhibitor of ROS secreted by *B. cinerea* could be reduced using plants over expressing a fungal oxalate decarboxylase of *Trametes versicolor.* Infection of such plants resulted in a faster ROS accumulation and resistance to *B. cinerea* than that observed in untransformed controls, demonstrating the importance of fungal suppression of ROS formation by oxalate. Thus, changes in the diffusive properties of the cuticle are linked with the induction ROS and attending innate defense.

Identification of grapevine pattern recognition receptors involved in PAMP-triggered immunity against *P. viticola* and *B. cinerea*

Presenting author: **Lucie Trda** (lucie.trda@gmail.com)

Authors: Trda L[1], Kelloniemi J[1], grapevine transformation platform[2] , Boutrot F[3], Heloir MC[1], Daire X[1], Wendehenne D[1], Zipfel C[3], Poinssot B[1]

[1]UMR Plante-Microbe-Environnement INRA 1088/ CNRS 5184/ Université de Bourgogne, 17 rue Sully, BP 86510, 21065 Dijon Cedex, France
[2]Plateforme de transformation Vigne, UMR Santé de la Vigne et Qualité du Vin, INRA/ Université de Strasbourg, 28 rue de Herrlisheim, BP 20507, 68021 Colmar Cedex, France
[3]The Sainsbury Laboratory, Norwich Research Park, Norwich, NR4 7UH, United Kingdom

Pattern-recognition receptors (PRRs) play a key role in plant immunity by assuring recognition of pathogen-associated molecular patterns (PAMPs), which are conserved molecular signatures of whole microorganism classes. PAMP perception constitutes the first layer of pathogen detection and activates defence mechanisms that aim to block pathogen development. It has been shown that PRR invalidation leads to reduced disease resistance.

Cultivated grapevine (*Vitis vinifera*) is highly susceptible to many diseases such as gray mould (*Botrytis cinerea*) or downy mildew (*Plasmopara viticola*). Several PAMPs, e.g. a beta-glucan laminarin, have been shown to induce defence responses and resistance to *P.viticola* and *B. cinerea* in grapevine. However, no corresponding PRRs are known so far in this crop. Our study aims to identify the orthologs of known PRRs in grapevine and investigate their role in the resistance to *P. viticola* and *B. cinerea*.

We have tested the responsiveness of grapevine to several known PAMPs and identified flg22 and chitosan as the elicitors of early signaling events in grapevine, including changes in free cytosolic calcium, production of reactive oxygen species or MAPK activation. In addition, both PAMPs trigger resistance against *B. cinerea* and *P. viticola*. Given the availability of grapevine genome we could identify in silico putative orthologs of *At*FLS2, *At*CERK1, *Os*CEBiP and *Mm*Dectin1 that might function as PRRs for flg22, chitin/chitosan and laminarin, respectively. Their functional analyses will be determined, firstly, by complementation assays in *A. thaliana* mutants and, secondly, by silencing strategy in transgenic grapevine cell suspensions and *in vitro* plants. Beside the PAMP-triggered resistance to *P. viticola* and *B. cinerea*, the basal disease resistance of silenced grapevine plantlets will be investigated.

SignWALLing: Signals derived from Arabidopsis cell wall activate specific resistance to pathogens

Presenting author: **Antonio Molina** (antonio.molina@upm.es)

Authors: Eva Miedes[1], Marie Pierre Riviére[1], Andrea Sánchez-Vallet[1], Clara Sánchez-Rodríguez[1], Magdalena Delgado[1], Philippe Ranocha[2], Xavier Bartel[3], Yves Marco[3], Deborah Goffner[2] and Antonio Molina[1]

[1]Centro de Biotecnología y Genómica de Plantas (UPM-INIA), Departamento Biotecnología-UPM, Campus Montegancedo, M40 (Km. 38). Universidad Politécnica Madrid. 28223-Pozuelo de Alarcón, Madrid, SPAIN.
[2]Unité Mixte de Recherche Centre Nacional de la Recherche Scientifique Univ Toulouse III, Pôle de Biotechnologie Végétale, BP 42617 Auzeville 24, Chemin de Borde Rouge, 31326 Castanet Tolosan, FRANCE.
[3]Laboratoire de Interactions Plantes-Microorganismes, Centre Nacional de la Recherche Scientifique Instiut National de la Recherche Agronomique, Chemin de Borde Rouge, 31326 Castanet Tolosan, FRANCE.

Plant cell walls constitute the first barrier that some types of pathogens, such as necrotrophic fungi, must overcome to colonise plant tissues. The traditional view of the cell wall as a passive barrier has evolved to a new concept that considers the wall as a dynamic structure that regulates both constitutive and inducible plant defence responses. The activation of plant innate immune system is triggered by microbe-associated molecular patterns (MAMPs) from the pathogens, but also can be regulated by damaged-associated molecular patterns (DAMPs) that are molecules released from plant cell walls upon pathogen infection. In line with this putative function of the cell wall in innate immunity, we have identified novel regulators (ELK2, AGB1 and ER [1, 2]) of *Arabidopsis* resistance to necrotrophic fungi that may also be involved in the control of cell wall integrity/architecture. To further characterize the function of cell wall on the regulation of immune responses we have performed a biased resistance screening of 100 putative/characterized primary/secondary *Arabidopsis* cell wall mutants. In this screening we have identified 20 mutants with altered susceptibility/resistance, compared to wild type plants, to at least one of the following pathogens: *Plectosphaerella cucumerina* (necrotrophic fungi), *Ralstonia solanacearum* (vascular bacterium), *Hyaloperonospora arabidopsidis (*biotrophic oomycete) and to a biotrophic powdery mildew fungus. Expression analyses of immune response genes in the selected mutants have revealed the complexity of the regulation of the defense responses in these mutants. Cell wall extracts from selected mutants a their capacity of activate resistance to particular pathogens was tested on wild-type plants. Interestingly, we found some wall extracts that induced resistance, further indicating the presence of signaling molecules in the wall extracts of the mutant tested. These data together with those obtained from the characterization of the cell wall structure/composition of the selected mutants suggest a putative interconnection between cell wall structure/composition and resistance/susceptibility to pathogens. These results will be used to build a cell wall topology map correlating specific wall modifications with plant resistance to particular type of pathogens.

Metabolic regulation of acquired resistance in Arabidopsis

Presenting author: **Jürgen Zeier** (Juergen.Zeier@uni-duesseldorf.de)
Authors: Navarova H, Ostler S, Bellwon P, Huber S, Zeier J
Institute for Plant Molecular Ecophysiology, Department of Biology, Heinrich-Heine University Düsseldorf, Germany

In their attempt to establish local (LAR) and systemic acquired resistance (SAR), plants respond to microbial pathogen attack with a whole battery of physiological and molecular responses. ATH1 microarray chip analyses show that pathogen-treated Arabidopsis plants undergo massive transcriptional reprogramming events both in inoculated and in distal, non-inoculated leaves. Upon localized *Pseudomonas syringae* pv. *maculicola* treatment, the transcript levels of 564 out of the 22800 genes on the ATH1 chip increase by a factor of 3 or more in leaves distant from the inoculation site. We denoted these genes as “SAR genes”, and alignments with publicly available microarray information associated with defense-, stress-, and hormone-related signaling showed that individual SAR genes are subject to distinct regulatory patterns and have allowed us to essentially group the 564 SAR genes in three main categories. Cluster I consists of 135 genes whose expression is independent of salicylic acid (SA)-signaling and includes critical SAR regulators such as ICS1, FMO1, ALD1, and PBS3. Cluster II is smaller than cluster I and contains about 55 genes that are tightly regulated by SA. Expression of the remaining genes, which were grouped in a third cluster, is partly but not totally SA-dependent. The transcriptional changes in plants in which SAR is biologically induced are virtually congruent with those of plants exogenously treated with the resistance enhancing “SA analog” BTH, implicating that BTH induces expression of both SA-dependent and SA-independent SAR genes. These observations in combination with recent metabolite analyses suggest that plant metabolites other than SA play a major role in the development of SAR, LAR, and β -amino butyric acid (BABA)-induced resistance. Beyond these findings, we will present novel aspects about the priming response associated with biologically-induced SAR in plants.

The Arabidopsis-*Bemisia tabaci* interactions: Deciphering plant responses and insect behaviors in susceptible and resistant genotypes

Presenting author: **Linda L. Walling** (linda.walling@ucr.edu)

Authors: Walling LL[1], Zhou J[1], Walker G[2], Zarate SI [1], Kempema L[1]

[1]Department of Botany and Plant Sciences, Center for Plant Cell Biology and Center for Disease-Vector Research, University of California, Riverside, CA 92521;

[2]Department of Entomology, Center for Disease-Vector Research, University of California, Riverside, CA 92521

Bemisia tabaci type B infests over 500 plant species, vectors over 100 viruses, depletes photosynthates, deposits copious amounts of honeydew that supports sooty mold growth, and causes developmental disorders to cause over a $ 1 billion in damage annually. However, few resistance genes have been identifies to control *B. tabaci*. Therefore, it is critical to identify the innate immune responses that protect plants from this phloem-feeding pest.

Changes in the global expression profiles based on microarray analyses in wild-type plants showed that *B. tabaci* nymph feeding induces salicylic acid (SA)-regulated and suppresses jasmonic acid (JA)-regulated defense signaling in *Arabidopsis thaliana*. Sentinel defense gene RNAs were quantified after *B. tabaci* infestation and revealed that responses to *B. tabaci* were biphasic. At early times after *B. tabaci* adult feeding, JA- and ethylene (ET)-responsive RNA levels increased and SA-responsive RNAs were repressed relative to non-infested plants. After 24 hr of feeding, there was a reprioritization of defense signaling. From 7 to 24 days after infestation, SA and SA-regulated RNAs accumulated locally and systemically. In accordance with the cross-talk between the JA and SA signaling pathways, JA- and ET-responsive gene RNAs declined or were unchanged in whitefly-infested leaves. Insect no-choice studies with defense mutants showed nymph development was accelerated in the mutants that activated SA-regulated or impaired JA-regulated defenses (*cim10* and *coi1,* respectively). Reciprocally, lines that activated JA-regulated (*cev1*) or impaired SA-regulated (*npr1,* NahG) defense gene expression slowed nymph development. Exogenous MeJA treatments indicated that JA-regulated defenses antagonize nymph development. Collectively, these data indicate that *B. tabaci* induces ineffective defenses and represses the effective JA-regulated defenses, which deter nymph development.

Electropenetration graph (EPG) studies have been used to understand the behaviors of whitefly adults and 2nd instar nymphs on wild-type plants, as well as the *cim10, coi1, npr1* and *cev1* defense mutants. The location of resistance determinants as revealed by EPG analyses will be discussed. Deep sequencing is being used to identify the gene cohorts that are correlated with the resistance or susceptibility expressed in defense mutants.

Insect eggs suppress plant defence against herbivory in Arabidopsis

Presenting author: **Philippe Reymond** (Philippe.Reymond@unil.ch)
Authors: Bruessow F[1], Gouhier-Darimont C[1], Reymond P[1]
[1]Department of Plant Molecular Biology, University of Lausanne, Switzerland

Insect egg deposition represents a threat for a plant, as larvae hatching from the egg will ultimately feed on their host. We found that oviposition by the butterfly *Pieris brassicae* triggers cellular and molecular changes that are similar to the changes caused by biotrophic pathogens in *Arabidopsis thaliana* and that the plant defense signal salicylic acid (SA) accumulates at the site of oviposition. This is unexpected since the SA pathway controls the defense against fungal and bacterial pathogens whereas it negatively interacts with the jasmonic acid (JA) pathway, which is crucial for the defense against herbivores. Application of *P. brassicae* or *Spodoptera littoralis* egg extract onto leaves reduced the induction of insect-responsive genes after challenge with caterpillars, showing for the first time that egg-derived elicitors suppress plant defense via the SA pathway. Consequently, larval growth of the generalist herbivore *S. littoralis*, but not of the specialist *P. brassicae*, was significantly higher on plants treated with egg extract than on control plants. These data revealed an intriguing facet of the crosstalk between SA- and JA-signaling pathways and suggest that insects have evolved a way to suppress the induction of defense genes by laying eggs that release elicitors. We are currently studying the nature of these elicitor(s) and how negative crosstalk is regulated at the molecular level.

(Z)-3-Hexenol emitted from common cutworm-infested tomato plants induces jasmonate-independent defence to the intact tomato plants against upcoming herbivore attack

Presenting author: **Koichi Sugimoto** (sugimok@ecology.kyoto-u.ac.jp)

Authors: Sugimoto K[1,2], Matsui K[1], Ozawa R[2], Iijima Y[3], Sasaki R[4], Akakabe Y[5], Akitake S[1], Nobuke T[1], Aoki K[4], Shibata D[4], and Takabayashi J[2]

[1]Graduate School of Medicine, Yamaguchi University

[2]Center for Ecological Research, Kyoto University

[3]Department of Nutrition and Life Science, Kanagawa Institute of Technology

[4]Kazusa DNA Research Institute

[5]Faculty of Agriculture, Yamaguchi University

Plants recognize the presense of herbivores indirectly by receiving the volatile compounds emitted from surrounding plants infested by the herbivores and fortify the defense systems for upcoming threats. Volatile-induced defense responses against herbivores regulated by jasmonic acid-dependent pathway are well studied in some plant speices, by contrast, little is known about defense system regulated by jasmonic acid-independent pathway. In this study, we report the novel defense pathway observed in (*Z*)-3-hexenol-exposed plants that is independent of jasmonic acid signals.

To clarify the molecular basis of defense induction, we used a tomato plant, *Solanum lycopersicum* cv. Micro-Tom, and common cutworm, *Spodoptera litura*, a herbivorous insect of wide range of plants. Intact tomato plants previously exposed to the volatiles from tomato plants infested by the common cutworm depressed the growth of common cutworm compared to those exposed to the intact tomato volatiles. Through the metabolomic analysis with LC-MS/MS, accumulation of a glycoside was detected in the tomato plants exposed to the common cutworm-infested tomato plant volatiles. When this glycoside was embedded in the artificial diet for common cutworm, the weight increase of the caterpillar was significantly depressed, indicating that the glycoside was one of the defense compounds in tomato leaves. This glycoside was also accumulated in *jasmonic acid insensitive* mutant plants after exposure to (*Z*)-3-hexenol, one of the constituents in the volatile blend emitted from common cutworm-infested tomato plants. The findings suggest that the (*Z*)-3-hexenol works as a defense induction compound in intact plants through the glycoside accumulation.

A root herbivore induces susceptibility to conspecifics in maize

Presenting author: **Christelle A. M. Robert** (christelle.robert@unine.ch)

Authors: Robert CAM[1], Hibbard B[2], Köllner T[3], Giron D[4], Body M[4], Villard N[1], Glauser G[1,5], Babst BA[6], Ferrieri RA[6], Zwahlen C[1], Turlings TCJ[1], Erb M[1,7*]

[1] University of Neuchâtel, Laboratory for Fundamental and Applied Research in Chemical Ecology (FARCE), Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland
[2] Plant Genetics Research Unit, 205 Curtis Hall, University of Missouri, Columbia MO 65211-7020, USA
[3] Department of Biochemistry, Max Planck Institute for Chemical Ecology, Beutenberg Campus, Hans-Knöll-Str. 8, 07745 Jena, Germany
[4] Physical Ecology and Multitrophic Interactions Group, Institut de Recherche sur la Biologie de l'Insecte, UMR CNRS 6035 UFR Sciences et Techniques, Avenue Monge- Parc Grandmont, 37200 Tours, France
[5] Chemical Analytical Service of the Swiss Plant Science Web, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland
[6] Medical Department, Brookhaven National Laboratory, Upton, NY 11973, United States of America
[7] Max Planck Institute for Chemical Ecology, Beutenberg Campus, Hans-Knöll-Str. 8, 07745 Jena, Germany
* Corresponding author: merb@ice.mpg.de

Plants have evolved a multitude of inducible strategies to protect themselves against their attackers. These strategies include various direct and indirect defense mechanisms as well as the reallocation of resources to storage organs. The inducible changes can lead to plant-mediated interactions between herbivores that share a common host plant. Here we report on a strong positive plant-mediated intraspecific interaction among larvae of the beetle *Diabrotica virgifera virgifera*, a major pest of maize roots in the USA and Europe. Field and laboratory experiments showed that the root herbivore performs better when aggregating on a host plant. Using a split root approach, we found that this effect cannot be attributed to conspecific stimulation by contact or by visual or volatile cues, but that it was purely plant-mediated. To investigate the possible underlying mechanisms of this induced susceptibility, we characterized plant defense chemistry, resource reallocation and primary metabolism. We detected no major effect on plant defense or resource reallocation, but we found that primary metabolism was strongly altered by herbivory, resulting in a sucrose and amino acids accumulation in roots, and enhancing the nutritional quality of roots to the herbivore. Unraveling the mechanism that allows *D. virgifera* to perform so well on maize may help to the development of novel maize varieties to fight the root pest.

Effect of nitrogen fertilisation of strawberry plants on the efficacy of defence-stimulating biocontrol products against *Botrytis cinerea*

Presenting author: **Philippe C. Nicot** (philippe.nicot@avignon.inra.fr)

Authors: Nicot PC[1], Bardin M[1], Lecompte F[2]

[1]INRA, UR407 Pathologie végétale, Domaine Saint Maurice, BP94, F-84143 Montfavet Cedex, France

[2]INRA, UR 1115 Plantes et Systèmes de Cultures Horticoles, Domaine Saint Paul, Site Agroparc, F-84914, Avignon Cedex

Nitrogen (N) fertilisation is known for its effect on the susceptibility of host plants to certain pests and pathogens and on the production of volatile secondary metabolites in response to attacks by herbivores. Although N is a key component in many compounds implicated in plant defence mechanisms, little is known on the possible effect of nitrogen fertilisation on the efficacy of defence-stimulating biocontrol agents. In the present work we examined the effect of five levels of N nutrition (0.5, 2, 5, 10 and 20 mMol L^{-1} applied as nitrate in a hydroponic system) on the susceptibility of strawberry leaves to *Botrytis cinerea* and on the protective efficacy of Serenade Max (*Bacillus subtillis* QST713) and Chitoplant (chitosan), two biocontrol products presumed to induce plant defence mechanisms.

Two days after the application of the products, batches of leaf disks were excised, inoculated with *B. cinerea* and incubated in conditions conducive to disease development. The resulting lesions were photographed and their surface was assessed with the help of image analysis software. Plant fertilisation had a highly significant ($p<0.01$) effect on disease severity for both strains of *B. cinerea* tested, with lesion sizes smallest and largest, respectively, on leaves from plants with the lowest and highest N levels. Similar effects were observed with plants treated with either biocontrol product.

In addition, plant fertilisation significantly influenced the efficacy of the biocontrol products. Compared to the untreated control, Serenade Max provided significant protection against both strains of *B. cinerea* on plants with low levels of N fertilisation (0.5 and 2 mMol L^{-1}), but not on those that received higher doses. In contrast, Chitoplant did not provide any significant protection against aggressive strain BC1. It provided a high level of protection (greater than 50%, $p<0.01$) against mildly aggressive strain BC21, but only for plants with low N fertilisation levels (0.5 and 2 mMol L^{-1}).

Possible hypotheses and the relevance of these results for integrated protection will be discussed.

Time effectiveness of using inducers of chemical and biological origin against powdery mildew (*Blumeria graminis* f. sp. *tritici*) on wheat

Presenting author: **Lubomir Vechet** (vechet@vurv.cz)

Authors: Vechet L

Crop Research Institute, Prague – Ruzyne, The Czech Republic

In three years of small-plot experiments the set of substances of chemical (BTH–benzothiadiazole, Bion ®; SA–salicylic acid; GB–glycine betaine) and biological origin– extracts from different parts of plants (OB–oak bark, Q*uercus robur* L.; GK giant knotweed, *Reynoutria sachaliensis* (F. Schmidt) Nakai; CU–curcuma, *Curcuma longa* L.; GI–ginger, *Zingiber officinale Roscoe*; SN–stinging nettle, *Urtice dioica*; IG–impatiens glandilufer, *Impatiens de L´Himalaya*) were used as an inducers of wheat resistance to powdery mildew on the cultivar Kanzler susceptible to this disease. Inducers were applicated on plants in the form of crop-spraying. In the year 2008 were used three terms of inducers application. The first treating have been carried out in the 6th December 2007, the second treating in the 28th April 2008, and the third treating in the 15th May 2008. In the year 2009 were used three terms of inducer application, too. The first treating have been made the 2nd December 2008, the second treating in 1st April, and the third treating in the 25th May. In the year 2010 have been carried out only the fist treatment in the 7th December 2009. Disease severity of powdery mildew was expressed as cumulative percentage of leaf area diseased (CPLADF) where values of the disease are expressed as disease severity of the all live leaves on the plant. Evaluation of the disease severity was carried out in 5-6 June of the year 2008, 15-17 June in the year 2009, and 10-11 June in the year 2010. In the year 2008 were disease severity of the cultivar Kanzler after treatments by individual inducers in three different terms was in average lower than on untreated control. Disease severities were in all cases lower than in the control except after the first treatment by GB where the value was higher than CO. The treatments in three different terms by BTH had the lowest disease severity in average and the highest values in average were after treatment by GB. The lowest disease severity was after the second treatment by BTH and GK. The lower values of the disease were after the first treatment in CU and GI than in further treatments. The high values of the disease after the first treatment than in further were in BTH and GB. The second treatments by inducers were higher than in others in CU and GI. Minimal differences in disease severity among individual terms of treatment were in OB. In the year 2009 was higher occurrence of the disease than in 2008. The value of the second treatment with SA was higher than in untreated CO. All others terms of treatments by individual inducers had lower disease severity than CO. The lowest average value of disease severity in three terms of treatment had BTH but the highest disease severity in average was after treatment by SA. The lowest disease severity after the first treatment was in SA and GB. On the contrary the highest values after the first treatments were in GK and CU. Small difference among individual terms of treatment in the same inducer were in OB and GI. In the year 2010 was made the first treatment only. All inducers showed lower disease severity than CO. The lowest values of the powdery mildew were after treatment by GK, GI and BTH. The highest disease severity was after treatment by GB. Obtained results showed long term effectiveness of inducers resistance tested in natural conditions.

What tools used to acquire a better understanding of induced resistance after elicitation and/or infection from laboratory to field experiments?

Presenting author: **Marie-France Corio-Costet** (coriocos@bordeaux.inra.fr)

Authors: Dufour MC[1], Lambert C[2], Cluzet S[2], Corio-Costet MF[1]

[1]INRA, UMR Santé et Agroécologie du Vignoble (SAVE-1065), Institut des Sciences de la Vigne et du Vin (ISVV), BP 81, 71 rue Bourlaud, 33883 Villenave d'Ornon, France

[2]ISVV, GESVAB-EA3675, CS 50008, 33882 Villenave d'Ornon, France

Plant defence efficacy results of interactions of various factors, including physiological and genetic characteristics of both partners in host-pathogen interaction as well as environmental conditions. Numerous studies showed an excellent efficacy of elicitors in laboratory conditions, but *in natura* the obtained results are often disappointing.

To acquire a better understanding of grapevine defence status in vineyard, after elicitor applications with benzothiadiazole or phosphonates, we have developed a triple approach (biological, biochemical and molecular) in our laboratory.

From experiments performed in laboratory, we choose a set of selected genes potentially involved in grapevine defense. After an assessment to estimate their interest as markers of grapevine defenses by RT-qPCR at different times, we have also assessed the impact of pathogen variability intra or inter species on the efficacy of elicitors. Finally, transcripts were specifically up or down regulated in infected-leaves according to elicitors or the intra and inter specific variability of pathogen. To complete the transcript approach, quantitative analysis of polyphenols and biological efficacy were also performed to correlate the different approaches to the efficacy of grapevine defenses. So we have expression patterns and polyphenol profiles of infected-leaves that could be use as potential markers to vineyard experiments.

In 2009 and 2010, these markers have been tested to estimate the defense level of molecules as benzothiadiazole, a phosphonate, and mancozeb (fungicide reference), after *Plasmopara viticola* artificial inoculation in vineyard. The samplings, the analyses, the epidemiological follow-up and the quality of the grape harvested showed that the grapevine defenses were correlated well at the level of protection and that some compounds have an effect on the development which seem directly connected to climatic and vintage year. Surprisingly the better up-regulation of transcripts was obtained with the fungicide mancozeb.

To date we thus have tools allowing to understand how reacts the plant in its agronomic environment, which should allow us to exploit and to set up better the use of elicitors in alternative or complementary strategies of grapevine pest management.

Diversity in susceptibility of *Botrytis cinerea* to biocontrol products inducing plant defence mechanisms

Presenting author: **Marc Bardin** (marc.bardin@avignon.inra.fr)

Authors: Bardin M, Comby M, Lenaerts R, Nicot PC

INRA, UR407 Pathologie Végétale, BP 94, 84140 Montfavet, France

The development of plant defence stimulants to increase host resistance represents an attractive alternative to fungicides for the protection of crops against plant pathogens. Various biotic and abiotic agents have been shown to induce defence mechanisms to various plant pathogens in different plant species. In this study we evaluated the efficiency of various biocontrol products (microorganisms, plant extracts and organic products) presumed to induce plant defence mechanisms against *Botrytis cinerea* on tomato and lettuce.

Two days after the application of the products, tomato and lettuce leaves were inoculated with *B. cinerea* and incubated in conditions conducive to disease development. The resulting lesions were photographed two days after inoculation and their surface was assessed with the help of image analysis software. Out of 9 products tested, Serenade Max (*Bacillus subtilis* QST713) proved to have a significant protective efficacy against the mildly aggressive strain of *B. cinerea* BC21 on both plants.

To assess the presence of low susceptibility to Serenade Max in populations of *B. cinerea*, the protective efficacy of this product was evaluated against 20 strains differing in their geographic origin, host of isolation and level of aggressiveness. To this end, tomato and lettuce leaves were treated with two concentrations of Serenade Max (0.2% and 0.8%), two days before inoculation. The efficiency of the product was significantly influenced by the isolate of *B. cinerea* tested (ANOVA, $p<0.01$ at both concentration of Serenade Max). It was more efficient when applied at a concentration of 0.8%, providing protection levels ranging from 45% to 85% on tomato leaves. No correlation was observed between the level of aggressiveness of *B. cinerea* strains and the protection provided by the biocontrol agent. Possible implications of these findings will be discussed.

Abietane diterpenoid in plant defense signaling

Presenting author: **Jyoti Shah** (shah@unt.edu)

Authors: Chaturvedi R[1], Venables BJ[1], Petros R[2], Takemoto LJ [3], Nalam V[1], Maoyin L[4], Wang X[4], and Shah J [1]

[1]Department of Biological Sciences, University of North Texas, Denton, TX, USA
[2]Department of Biological Sciences, University of North Texas, Denton, TX, USA
[3]Division of Biology, Kansas State University, Manhattan, KS, USA
[4]Department of Biology, University of Missouri and Donald Danforth Plant Science Center St. Louis, MO, USA.

Abietane diterpenoids are major constituents of conifer resins that have important industrial and medicinal applications. Abietane diterpenoids are also synthesized by angiosperms. However, their function in plants is poorly understood. Here we show that dehydroabietinal (DA), an abietane diterpenoid, is one of the most potent activators of systemic acquired resistance (SAR), which is an inducible defense mechanism that is activated in the distal organs of a plant that has experienced a local foliar infection. DA was purified as a SAR-activating factor from the vascular sap of *Arabidopsis thaliana* leaves treated with a SAR-inducing microbe. Locally applied DA is translocated through the vasculature and systemically induces the accumulation of salicylic acid (SA), an important activator of defense, thus leading to enhanced resistance against subsequent infections. The *NPR1* (*NON-EXPRESSOR OF PR GENES1*), *FMO1* (*FLAVIN-DEPENDENT MONOOXYGENASE1*) and *DIR1* (*DEFECTIVE IN INDUCED RESISTANCE1*) genes, which are critical for biologically-induced SAR, are also required for the DA-induced SAR, which is further enhanced by azelaic acid, a defense priming molecule. In response to the biological induction of SAR, DA in the vascular sap is redistributed into a SAR-inducing high molecular weight complex that is sensitive to trypsin, thus suggesting that DA orchestrated SAR involves a vascular sap protein(s).

Differential effects of biotic and abiotic elicitors on plant defence and physiology in radiata pine seedlings

Presenting author: **Tony Regliński** (tony.reglinski@plantandfood.co.nz)

Authors: Regliński T[1], Taylor JT[1], Gould N[1], Northcott GN[1], Spiers M[1], Ah Chee A[1], Rodenburg N[2], Hill RA[3]

[1]The New Zealand Institute for Plant & Food Research Limited, Hamilton, New Zealand.

[2] Department of Biology, Utrecht University, The Netherlands.

[3] Bio-Protection Research Centre, Lincoln University, Christchurch, New Zealand.

Radiata pine (*Pinus radiata*) seedlings exhibited elevated resistance to stem infection by *Diplodia pinea* after foliar spray with 1mM methyl jasmonate (MeJA). Induced resistance was transient, being greatest one to two weeks after MeJA application and was accompanied by reduced seedling growth. This is consistent with hypotheses describing trade-offs in resource allocation between growth and defence and, in this instance, may be attributable to reduced carbon fixation and/or mobilization of carbon from the needles to the stem and roots. Increasing the MeJA concentration and/or application frequency did not improve disease control efficacy. Furthermore, it caused a dose-dependent reduction in photosynthetic activity and needle water potential, resulting in an increase in the dry weight: fresh weight ratio. At high doses, MeJA was phytotoxic and caused needle chlorosis and severe stunting or even death.

Root drench application of *Trichoderma atroviride* promoted the growth of radiata pine seedlings and induced systemic resistance to stem infection by *D. pinea.* When used in combination with MeJA, *T. atroviride* root drench did not prevent the retardation of seedling growth caused by MeJA, nor was there any significant additive benefit with regard to the phenotypic resistance to *D. pinea* stem infection. However, there was biochemical evidence of a synergistic treatment effect on inducible host defences including terpenoids, phenolics and pathogenesis-related proteins. For example, peroxidase activity and concentrations of α-pinene and β-pinene were greater in seedlings treated with *T. atroviride*+MeJA than in seedlings treated with the component parts alone. Conversely, the activity of phenylalanine ammonia lyase, a key enzyme in phenolics biosynthesis, was suppressed in the treated seedlings, indicating some form of trade-off between phenolic and terpenoid defence pathways.

Irradiation of radiata pine seedlings with ultraviolet light (UV-C, 254 nm) has been shown to induce a dose-dependent resistance against *D. pinea* stem infection. UV-C enhanced activity of defence-related enzymes but did not affect the concentrations of α-pinene and β-pinene in treated seedlings.

The differential responses of radiata pine to biotic and abiotic elicitors demonstrates the complexity of induced resistance and affirms the need to better understand this phenomenon in order to maximise its potential for disease management.

Specific phosphorylation of conserved eukaryotic protein SGT1 is an essential element of plant defense against pathogens

Presenting author: **Magdalena Krzymowska** (krzyma@ibb.waw.pl)

Authors: Krzymowska M, Hoser R, Zurczak M, Hennig J

Institute of Biochemistry and Biophysics, PAS, Warsaw, Poland

Salicylic acid - induced protein kinase (SIPK), a MAP kinase that is rapidly activated in response to pathogen challenge is involved in induction of defense-related genes and HR cell death. Transgenic tobacco plants with manipulated levels of SIPK protein display a range of phenotypic abnormalities and contain altered levels of phytohormones compared to wild-type plants. The ubiquitin ligase-associated co-chaperone protein SGT1 has been shown to be required for plant immune responses, cell death and hormone signaling. Thus, SGT1 could provide a possible link between the modified SIPK levels and the pleiotropic phenotypic changes observed in the transgenic plants. To test whether SGT1 is a SIPK substrate, we performed transient co-expression of SGT1-Strep-tag II, CA-NtMEK2 and SIPK in *Nicotiana benthamiana*. Mass spectrometric (LC-MS-MS/MS) analysis of affinity purified SGT1 revealed that it is phosphorylated on Ser^{358} within a canonical MAPK target-SP-motif. Heterologous transient complementation assays were used to study the significance of this phosphorylation in the ability of tobacco plants to mount effective defense against TMV infection. Changes in the subcellular distribution of SGT1 phosphovariants have been investigated by confocal laser microscopy. The phospho-mimic variant of SGT1 showed highly increased nuclear accumulation in comparison to wild-type or phospho-null proteins, consistent with the model that phosphorylation can facilitate movement of SGT1 into nucleus. Our data indicate that post-translational modification of SGT1 is crucial for its function in controlling plant defense responses.

The hemibiotrophic fungus *Colletotrichum graminicola* triggers above- and belowground systemic resistance in *Zea mays*

Presenting author: **Dirk Balmer** (Dirk.Balmer@unine.ch)

Authors: Balmer D, Planchamp C, Villacres de Papajewski D, Mauch-Mani B

University of Neuchâtel, Laboratory of Molecular and Cell Biology, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

Upon localized foliar infections by diverse pathogens, plants often develop a whole plant immunity called systemic acquired resistance (SAR). During this process, plants generate a series of mobile defense signals that move from the infected tissue to distal parts, thus inducing a broad-spectrum systemic resistance to subsequent pathogen attack. A significant research progress over the past few years led to the discovery of several key components of the SAR signalling mechanism. However, the vast majority of SAR research has been performed on dicotyledonous plants such as *Arabidopsis* or tobacco. Studies of systemic pathogen defense capacities in monocots are scarce. Moreover, SAR research has been traditionally focused on leaf-to-leaf signalling, whereas studies on belowground systemic resistance are rare. Therefore, our project aims to dissect above- and belowground systemic resistance in maize, focusing also on shoot-to-root and *vice versa* signalling. We are analyzing systemic defense reactions triggered by the hemibiotrophic fungus *Colletotrichum graminicola*. *C. graminicola* is able to infect both maize leaves and roots, which makes it convenient for the analysis of both leaf and root systemic defense reactions. We performed an extensive local and systemic transcriptional profiling upon leaf and root infections. Similar gene expression patterns in infected leaves and roots were detected, although the roots responded faster than the leaves but exhibited generally much lower levels of defense-related gene expression. We also detected an adaptation of gene expression in systemic leaves upon leaf infection. Intriguingly, root infections triggered an extensive transcriptional reprogramming of defense-related genes in the leaves. We also observed that pre-infections of second maize leaves with *C. graminicola* triggered a systemic resistance against the same fungus in the third, younger leaves. We are currently performing a metabolomic analysis to find local and systemic defense compounds, and to determine which hormonal pathways are involved in shoot-to-root and root-to-shoot respectively, systemic defense signalling.

Characterization of *Trichoderma harzianum* T39-induced resistance of grapevine against downy mildew

Presenting author: **Michele Perazzolli** (michele.perazzolli@iasma.it)

Authors: Perazzolli M[1], Palmieri MC[1], Roatti B[1,2], Moretto M[1], Fontana P[1], Ferrarini A[3], Velasco R[1], Delledonne M[3] and Pertot I[1]

[1]IASMA Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, S. Michele all'Adige 38010, Trento, Italy

[2]Institute of Integrative biology, Swiss Federal Institute of Technology (ETH), 8092 Zurich, Switzerland

[3]Università degli Studi di Verona, Dipartimento di Biotecnologie, Strada Le Grazie 15, 37134 Verona, Italy

Downy mildew, caused by the oomycete *Plasmopara viticola*, is one of the most destructive grapevine diseases. Its control is based on frequent chemical treatments, but concerns about health and environmental impact of pesticide overuse have made alternatives increasingly attractive. Enhancement of plant resistance by natural resistance inducers seems to be a promising strategy for controlling crop diseases, but scarce information is available on the molecular mechanisms and energy cost of induced resistance in non-model plants.

Our aim is to characterize the resistance mechanisms activated in grapevine by the biocontrol agent *Trichoderma harzianum* T39 (T39) in order to identify genes and processes activated for defence against *P. viticola* infection.

T39 treatments significantly reduced downy mildew symptoms without any direct toxic effect on *P. viticola* sporangia, by activating grapevine resistance, both locally and systemically. Local effect of T39 was comparable to the standard copper treatment under controlled greenhouse condition. Repeated T39 applications did not affect grapevine growth, shoot and root weight, leaf dimension and chlorophyll content, indicating absence of apparent energy cost for resistance activation. Expression analysis of marker genes suggested the involvement of jasmonic acid and ethylene signals in the T39-induced resistance.

Trancriptomic analysis of T39-induced resistance was performed using the RNA-Seq protocol followed by next generation sequencing by Illumina. About 25 millions of 36 bp-long reads were obtained form control and T39-treated leaves, collected before or 24 h after *P. viticola* inoculation. Reads mapping to grapevine genome were used to estimate gene expression, and a complex transcriptional reprogramming during T39-induced resistance was detected. Particularly, T39 induced the expression of grapevine genes in the absence of pathogen infection and reinforces the expression of other genes after *P. viticola* inoculation. Interestingly, T39-treated plants showed a specific up-regulation of defence-related processes after *P. viticola* inoculation and attenuated modulation of genes repressed by downy mildew in control plants. Annotation of modulated genes revealed the activation of signal transduction, transcription and defence functional categories, indicating a potential key role of these genes in the activation of grapevine resistance. Further characterization of some regulatory genes will identify processed that could be enhanced to naturally increase the grapevine resistance.

Information war in the plant headspace

Presenting author: **André Kessler** (ak357@cornell.edu)
Cornell University, Department of Ecology & Evolutionary Biology, Ithaca, NY, USA

Plants respond to insect herbivore damage with an array of changes in primary and secondary metabolism. Some of these metabolic changes can cause increased resistance of the plant to current and subsequent herbivore attack and may function as direct and indirect defences. Such induced resistance effects have been extensively studied but very little is known about their role in structuring arthropod communities, influencing insect herbivore population dynamics, and conversely the distribution of herbivory within plant populations.

I will discuss ecological consequences of plant defences and evaluate the resulting costs and benefits of induced plant responses. In particular I will focus on herbivore-induced volatile organic compound (HIVOC) -mediated effects on insect community dynamics and interactions with plant mutualists, such as pollinators and natural enemies of herbivores.

Results from two major study systems, wild tomatoes, *Solanum* spp., from the pacific slope of the Andean Mountains and goldenrod, *Solidago altissima*, from north-eastern North America as well as a literature review suggest that indirect defence is rarely a primary function of HIVOC emissions. For example, herbivore-induced volatiles can function more directly by affecting choice and dispersal of herbivores in goldenrod. On the other hand HIVOCs can result in significant fitness costs for wild tomato plants without obvious benefits. Herbivore-induced changes in floral volatile emission cause pollinators of the wild tomato *Solanum peruvianum* L. to avoid flowers on damaged plants resulting in herbivore-induced pollinator limitation, an ecological cost of induced resistance in general and HIVOC emission in particular.

The above examples have major implications on our understanding of the role of herbivore-induced plant responses for structuring arthropod communities and in affecting herbivore dispersal and plant fitness. Similarly, they can provide guidance for an objective analysis of herbivore-induced plant responses as potentially useful traits in agriculture. Molecular and chemical ecology studies into the underlying mechanism on native study systems are crucial to make these evaluations.

Multi-species multitrophic plant-insect interactions: from ecology to genes

Presenting author: **Roxina Soler** (roxina.soler@wur.nl)

Authors: Soler R and Dicke M

Laboratory of Entomology, Wageningen University, P.O. Box 8031, 6700 EH Wageningen, The Netherlands

Plants face a variety of herbivorous insects and can use induced responses to defend themselves against these attackers. Induced responses are mediated by signal-transduction involving phytohormones, such as jasmonic acid (JA) and salicylic acid (SA). In linear bi-trophic systems, interactions between JA and SA signalling pathways have been proposed to allow plants to fine-tune their defences depending on the feeding guild of the attacker. A central question in this emerging field is to understand how responses to single attackers interfere with responses to other attackers, especially by integratively addressing molecular and ecological aspects. I will show you a case study where insects from contrasting feeding guilds can benefit from the distinct pathways that are triggered in the plants by them, representing a constraint for the plant when sequentially attacked by phloem feeders and leaf chewers. The model system in our study consists of the brassicaceous plant *Brassica oleracea* (Brassicaceae), two common herbivores that feed on *Brassica spp.* and that coexist in time during most of the development of the plant the leaf chewer *Pieris brassicae* (Lepidoptera: Pieridae) and the phloem feeder *Brevicoryne brassicae* (Homoptera: Aphididae), and their respective main koinobiont parasitoids: *Cotesia glomerata* (Hymenoptera: Braconidae) and *Diaeretiella rapae* (Hymenoptera: Aphidiidae). We addressed the performance of the two herbivores, and their respective parasitoids, under single and multiple infestation. We analyzed the underlying defense mechanisms in the plant; levels of the phytohormones JA and SA, transcriptional responses of a number of selected defence-related genes, and secondary plant compounds were quantified at different time points during the single and multiple infestations. Subsequently, we explored whether, and how, such interactions can influence the oviposition and feeding preferences of the herbivores and parasitoids. Our data show that aphids and caterpillars interact via their host plant asymmetrically and the effects even translate to members of the third trophic level.

Symbiotic ants as an indirect defence against phytopathogens in an ant-plant mutualism

Presenting author: **Marcia González-Teuber** (mgonzalez-teuber@ice.mpg.de)

Authors: González-Teuber M[1], Heil M[2], Boland W[1]

[1]Department of Bioorganic Chemistry, Max Planck Institute for Chemical Ecology, Jena, Germany

[2]Departamento de Ingeniería Genética, CINVESTAV-Irapuato, Irapuato, Guanajuato, México

In obligate ant-plant mutualisms, host plants provide nesting space and food rewards for ants, whereas the main function of ants is assumed to be the defence against herbivores. A putative further function of symbiotic ants against pathogens has been suggested, nevertheless has not been empirically demonstrated so far. Using the obligate ant-plant *Pseudomyrmex-Acacia* mutualism as a study system, we tested the effect of the symbiotic ant *P. ferrugineus* on the community of leaf bacteria and the defensive physiology of their host plant, *A. hindsii*. Bacterial abundance increased significantly when the symbiotic ant was removed from the plant. Phytopathogen infection in *A. hindsii* led to changes in PR-protein enzyme activities: chitinases and peroxidases increased when plants were deprived from symbiotic ants. Chlorophyll content decreased, whereas the concentration of hexose in leaves increased, in response to phytopathogen infection (that is, when the defending ants were removed experimentally). Our results show that the protective service that is provided by symbiotic ants also covers the defence against phytopathogens, and that its absence affects the physiological defence state of the plants. As facultative ant-plant interactions are more common than symbiotic ones, future studies should consider whether the presence of ants on plants in general affects plant-pathogen infections.

Different systemic induced defense responses in roots and shoots after an above- or belowground jasmonic acid application

Presenting author: **Tom Tytgat** (t.tytgat@science.ru.nl)

Authors: Tytgat T[1], Verhoeven KJF[2], Jansen J[3], McIntire L[4], van der Putten WH[2,5], Biere A[2], van Dam NM[1]

[1]Department of Ecogenomics, Institute for Water and Wetland Research, Radboud University Nijmegen, Nijmegen, The Netherlands
[2]Department of Terrestrial Ecology, Netherlands Institute for Ecology, Wageningen, The Netherlands
[3]Swammerdam Institute for Life Science, University of Amsterdam, The Netherlands
[4]Department of Molecular Genetics & Microbiology, University of Florida, Gainesville, USA
[5]Department of Nematology, Wageningen University, Wageningen, The Netherlands

Upon wounding or attack by herbivores, plants respond by a rapid induction of defense pathways that are mainly controlled by the hormone jasmonic acid. Recently, it has been shown that jasmonic acid-induced gene profiles are largely context dependent [1]. Here, we analyzed the context dependency of the defense pathways in roots and shoots after an above- or belowground jasmonic acid application by gene transcript profiling and primary metabolite quantification. In shoots of a feral *Brassica*, a massive immediate change in primary and secondary metabolism was observed. In roots, the response was far less extensive and also qualitatively different from that in the shoots. The systemic defense response was also found to differ. Depending on whether the jasmonic acid had been applied below- or aboveground, in both roots and shoots we observed a different gene expression profile of genes involved in the synthesis of glucosinolates, protease inhibitors and volatiles. In conclusion we can state that the jasmonic acid response is not only tissue specific but also dependent on the location of the initial induction.

Pauwels, L., D. Inze, and A. Goossens, Jasmonate-inducible gene: what does it mean? Trends in Plant Science, 2009. 14(2): p. 87-91.

MYB8 transcription factor : a universal regulator of inducible phenolamides in *Nicotiana attenuata* after herbivory

Presenting author: **Nawaporn Onkokesung** (nawaporn.onkokesung@wur.nl)

Authors: Onkokesung N[1,#], Gaquerel E [1], Kotkar H[1], Kaur H[1], Baldwin IT[1], Galis I[1,&]

[1]Department of Molecular Ecology, Max Planck Institute for Chemical Ecology, Jena, Germany
[#] Laboratory of Entomology, Wageningen University, Wageningen, The Netherlands
[&] Institute of Plant Science and Resources Okayama University, Kurashiki Okayama, Japan

Phenolamides (PAs) are the diverse group of secondary metabolites that widely distributed in plant kingdom. Recent information showed that PAs accumulation were increased as a plant response to biotic (pathogen, insect herbivores) and abiotic (UV-irradiation, drought, mineral deficiency) stresses suggested the defensive role of PAs. In native tobacco (*Nicotiana attenuata*), accumulation of two major PAs, caffeoylputrescine (CP) and dicaffeoylspermidine (DCS) after herbivore attack is controlled by a key transcriptional activator MYB8, which is directly regulated by jasmonic acid signaling. Using a broadly- target metabolomics approach, we show that MYB8 is a master regulator of numerous PAs that consisting of hydroxycinnamic acid (caffeic acid, ferulic acid and ρ-coumaric acid) and two polyamines (putrescine and spermidine). We further identified the candidate MYB8-regulated genes from transgenic *N. attenuata* plant silencing in MYB8 activity (irMYB8) after herbivore attacked. Several candidates annotated as putative hydroxycinnamoyl transferases were identified and three candidates selected based on their expression patterns were cloned and characterized. One of the selected candidates is a gene encoding caffeoyl putrescineacyltransferase (AT1) which is an authentic CP synthase enzyme. The other two candidates namely DH29 and CV86 are encoding caffeoyl spermidineacyltransferase and caffeoyl caffeoylspermidineacyltransferase, respectively, which are essential for DCS biosynthesis. MYB8, AT1, DH29 and CV86 expression were strongly reduced in JA-deficient (asLOX3) plants after mimicking herbivory treatment which indicates that CP and DCS biosynthesis are directly regulated by JA signaling. Slightly different in herbivore performances fed on the plants silencing in either AT1, DH29 or CV86 activity suggests the overlapping function of CP and DCS in plant defense against tissue chewing herbivore. Our results show that a sole transcription factor can completely control one important metabolite branch (PAs). Moreover, we also be able to reconstruct the event from herbivory perception to metabolites reprograming as plant responds to herbivore stress.

Unravelling mycorrhiza induced resistance

Presenting author: **Maria José Pozo** (mariajose.pozo@eez.csic.es)

Authors: Pozo MJ, Jung SC, van Dam P, Martinez-Medina A, Fernández I, Torres R, García JM, López-Ráez JA and Azcón-Aguilar C

Department of Soil Microbiology and Symbiotic Systems. Estación Experimental del Zaidín-CSIC. Prof. Albareda 1, 18008 Granada, Spain

Arbuscular mycorrhizal (AM) associations are the most widespread and ancient plant-microbe symbiosis on Earth. The symbiosis significantly alters the host plant physiology, usually improving the plant ability to cope with biotic and abiotic stresses. Besides improved plant nutrition, experimental evidences support the involvement of plant defences in this bioprotection. In fact, a mild, but effective activation of the plant immune responses seems to occur during root colonization by AM fungi. We propose that this activation leads to a primed state of the plant that allows a more efficient activation of defence mechanisms in response to the attack by potential enemies.

In the model system tomato-*Glomus mosseae*, hormonal and transcriptional profiling pointed out the regulation of jasmonate (JA) dependent responses during the AM interaction. Remarkably, mycorrhizal tomatoes displayed a primed response to exogenous application of JA. To analyze the role of priming of JA dependent defenses in Mycorrhiza Induced Resistance (MIR) against foliar pathogens, we studied the interaction of tomato with *Botrytis cinerea*. Disease severity and pathogen proliferation was significantly reduced in *G. mosseae* colonized plants, while the expression of JA-marker genes upon *Botrytis* infection was higher. Moreover MIR was lost in tomato mutants impaired in JA signalling. All together, our results support that MIR is mediated by priming of JA-dependent responses. When looking for candidate molecules to be responsible for such primed response, we found in AM plants elevated basal levels of the peptidic hormone prosystemin (PS), a positive regulator of JA signalling in tomato. Experiments with the lepidopteran *Manduca sexta,* sensitive to PS regulated responses, confirmed enhanced resistance of mycorrhizal plants to this pest, supporting the role of PS in MIR. Remarkably, prosystemin antisense lines are unable to establish the symbiosis, thus PS appears to be a key element in AM regulation. Accordingly, we propose that regulation of PS is essential for AM establishment in tomato. Once the symbiosis is established, elevated PS basal levels in AM plants may act as amplifiers of JA regulated responses upon attack, leading to induced resistance.

Trichoderma induced plant responses to abiotic and biotic stimuli

Presenting author: **Ada Viterbo** (viterbo@agri.huji.ac.il)

Authors: Brotman Y[2], Landau U[1], Chet I[1], Viterbo A[1]

[1]Department of Plant Pathology and Microbiology, The Smith Faculty of Agriculture, Food and Environment, The Hebrew University of Jerusalem, Rehovot 76100 Israel

[2]Max-Planck-Institut für Molekulare Pflanzenphysiologie, Am Mühlenberg 1, 14476 Potsdam-Golm, Germany

Microarrays analysis of *Arabidopsis* roots inoculated with *Trichoderma asperelloides* T203 shows that the majority of the 280 transcripts which present a significant fold change (>2fold) are genes associated to plant growth and resistance to both biotic and abiotic stresses, including transcription factors with a central role in the crosstalk between these two pathways.

Trichoderma sp. are indeed versatile beneficial fungi which can stimulate plant growth and resistance to diseases and also plant tolerance to a wide range of adverse environmental conditions.

Soil treatment with a *T. asperelloides* spores suspension (10^6/g soil) was found to improve seeds germination under salt stress, both in *Arabidopsis* and cucumber.

The pool of reduced ascorbic acid is significantly increased in *Trichoderma* treated cucumber plants as the expression of catalase *(cat)* and Mn/Cu-dependent superoxide dismutase *(SOD*) genes. 1-Aminocyclopropane-1-carboxylate (ACC)-deaminase silenced *Trichoderma* mutants cannot provide tolerance to salt stress, suggesting that *Trichoderma*, similarly to ACC deaminase producing bacteria, can ameliorate plant growth under abiotic stressful conditions by lowering deleterious elevated ethylene levels accompanied by an elevated antioxidative capacity.

Jasmonates in biotic interactions of *Medicago truncatula* roots

Presenting author: **Bettina Hause** (bhause@ipb-halle.de)

Authors: Hause B, Heumann J, Hilou A, Landgraf R, Mrosk C, Schaarschmidt S

Leibniz Institute of Plant Biochemistry, Halle/Saale, Germany

Jasmonic acid (JA) and its derivatives, commonly termed jasmonates, are hormonal regulators involved in plant responses to abiotic and biotic stresses as well as in plant development. Jasmonates are lipid-derived signals synthesized via the allene oxide synthase branch of the so-called lipoxygenase pathway. The role of jasmonates is well established as part of a complex signal transduction pathway activated upon wounding of leaves by insects or upon interaction of plants with microorganisms. Among these interactions, also the mutualistic interactions between plants and arbuscular mycorrhizal (AM) fungi or nitrogen-fixing rhizobacteria are believed to be regulated by different plant signals including JA. To get deeper insights for JA, functional analyses by transgenic approaches were performed to investigate its role during the interaction between *Medicago truncatula* and *Glomus intraradices, Shinorhizobium meliloti* or *Aphanomyces euteiches*.

The capacity of *M. truncatula* roots to synthesize JA was changed by transformation with *A. rhizogenes* leading to chimeric plants. This was achieved by modulation of the transcript level of the *MtAOC1* gene encoding the allene oxide cyclase (AOC), a crucial enzyme involved in JA biosynthesis. Transgenic roots exhibiting partial suppression of *MtAOC1* and lower JA levels showed a significant delay in the process of colonization with *G. intraradices*. In contrast to mycorrhization, a role of JA in the interaction of *M. truncatula* with *S. meliloti* leading to the formation of nodules could not be demonstrated. Here, overexpression and partial suppression of *MtAOC1* did not lead to an altered nodule phenotype: Neither the morphology of nodules nor the number of nodules was different in these plants in comparison to the empty vector control.

Wounding of plants leads to endogenous rise of JA accompanied by the expression of a distinct set of genes. Therefore, we addressed the question, how roots and shoots of *M. truncatula* respond to wounding and whether wounding of leaves affects the biotic interactions of *M. truncatula* roots. Subsequent wounding of leaves resulted in enhanced JA levels corresponding to priming by JA. After repeated wounding colonization with the AM fungus was increased, whereas the interaction with the rhizobacterium was not affected. In contrast, the infection with the root pathogen was reduced, although accompanied by a synergy of the negative effects of wounding and infection. In conclusion, wounded plants appeared to be less susceptible to pathogens, which might be caused by JA-induced defense mechanisms, whereas positive effects of leaf wounding on mycorrhization point to jasmonates as positive regulators of formation of AM. In addition to JA itself, the positive effect might be mediated by systemically induced cwINV, previously shown to exhibit a regulatory function on AM.

The bacterial N-acyl homoserine lactone oxo-C14-HSL from the tripartite *Piriformospora* symbiosis confers disease resistance via activation of AtMPK6 and AtMPK3

Presenting author: **Karl-Heinz Kogel** (karl-heinz.kogel@agrar.uni-giessen.de)
Authors: Schikora A, Schenk S, Stein E, Kogel KH
Justus Liebig University Giessen, Research Center for BioSytems, Land Use and Nutrition

Quorum sensing (QS) mechanisms are crucial for bacterial populations to establish pathogenic or mutualistic interactions with their eukaryotic hosts. Many Gram-negative bacteria use N-acyl homoserine lactones (AHLs) as signals in such communication. Here we show that plants perceive AHLs and that the length of acyl moiety and the functional group at the y-position specify the plants response. Root treatment of Arabidopsis or barley plants with the long chain N-3-oxo-tetradecanoyl-L-homoserine lactone (oxo-C14-HSL) resulted in enhanced resistance of either plant to the respective powdery mildew fungi and in case of Arabidopsis also to *Pseudomonas syringae* pv. *tomato* DC3000. oxo-C14-HSL promoted a prolonged activation of AtMPK3 and particularly AtMPK6 when additionally challenged with flg22, followed by a higher expression level of defense-related transcription factors WRKY22 and WRKY29. In contrast to wild-type Arabidopsis, the mpk6 mutant was compromised in the AHL effect, suggesting that AtMPK6 is required for AHL-induced resistance. In this talk we will demonstrate that bacterial AHLs are critical in the plant's respond to rhizosphere communities. We will discuss agronomic issues and possible biotechnology strategies to exploit the activities of AHLs in plant production.

AM symbiosis as a control strategy against root parasitic plants through strigolactone reduction

Presenting author: **Juan A. Lopez-Raez** (juan.lopezraez@eez.csic.es)

Authors: Lopez-Raez JA[1], Torres-Vera R[1] , Kohlen W[2], Charnikhova T[2], Garcia JM[1], Bouwmeester H[2], Pozo MJ[1]

[1]Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín (CSIC), Granada, Spain

[2]Laboratory for Plant Physiology, Arboretumlaan 4, 6703 BD Wageningen, the Netherlands

Strigolactones (SLs) are a new class of plant hormones emerging as important signals in the control of plant architecture. Moreover, they are key elements in plant communication with several below-ground organisms. In the rhizosphere, the SLs act as host detection molecules for beneficial arbuscular mycorrhiza (AM) fungi, but also as germination stimulants for root parasitic plant seeds (Bouwmeester *et al.*, 2007). Parasitic weeds of the family Orobancheaceae - including *Striga*, *Orobanche* and *Phelipanche* spp - cause severe damage to important agricultural crops worldwide. The lifecycle of the root parasitic weeds is intimately associated with their host, especially at the very early stages of the interaction, being therefore a suitable target to develop new and more effective control strategies. Using tomato-AM fungi as a model system, we have recently shown that AM symbiosis induces changes in both transcriptional and hormonal profiles mainly related to the jasmonates (López-Ráez *et al.*, 2010). Moreover, we have analytically demonstrated that SL production is significantly reduced upon AM symbiosis (López-Ráez *et al.*, 2011) and that the SL-deficient tomato mutant *SlCCD8* reduced parasitic plant infestation to a higher extent than AM symbiosis. Considering the dual role of the strigolactones in the rhizosphere as signals for AM fungi and parasitic plants, we will discuss the potential implications of these changes in the plant interaction with both organisms.

Regarding the infection process, an induction of genes associated to the biosynthesis of jasmonates was observed in the host plant at the early stages of tomato infection by the parasitic plant *Phelipanche ramosa*. Conversely, a negative modulation of the salicylic acid (SA) pathway was envisaged, suggesting that a SA reduction is required for a successful parasitic infection.

Bouwmeester et al. (2007). Rhizosphere communication of plants, parasitic plants and AM fungi. Trends in Plant Science 12(5): 224-230.

López-Ráez et al (2010). Hormonal and transcriptional profiles highlight common and differential host responses to arbuscular mycorrhizal fungi and the regulation of the oxylipin pathway. Journal of Experimental Botany 61(10): 2589-2601.

López-Ráez et al. (2011). Arbuscular mycorhizal symbiosis decreases strigolactone production in tomato. Journal of Plant Physiology 168(3): 294-297.

How “innate” is the plant immune system?

Presenting author: **Jurriaan Ton** (jurriaan.ton@bbsrc.ac.uk)
Authors: Luna E[1,5], Ahmad S[1,4], Zhang Y[1], Bruce T[1], Flors V[2], Pastor V[2], Mauch-Mani B[3], Van Hulten M[4], Wees, SCM[4], Pieterse CMJ[4], Roberts M[5], Ton J[1,6]
[1]Rothamsted Research, Centre for Sustainable Pest and Disease Management, Harpenden, UK
[2]University of Jaume I, Department CAMN, Laboratory of Biochemistry and Plant Biotechnology, Castellon, Spain
[3]University of Neuchâtel, Department of Biology, Laboratory of Molecular and Cellular Biology, Neuchâtel, Switzerland
[4]University of Lancaster, Lancaster Environmental Centre, Lancaster, UK
[5]Utrecht University, Institute of Environmental Biology, Plant-Microbe Interactions, Utrecht, The Netherlands
[6]University of Sheffield, Department of Animal and Plant Sciences, Sheffield, UK.

Plants rely on their immune system to survive in hostile environments, where they are regularly threatened by harmful organisms. Although the plant immune system is often referred to as “innate”, it allows adjustment to different threat levels and types. Specific environmental stimuli can prime plant immune responses, causing a faster and/or stronger defence response to attacking pathogens and/or herbivores. For instance, the non-protein amino acid beta-aminobutyric acid (BABA) can prime various defence responses in the model plant species *Arabidopsis thaliana*. Using this model system, we discovered a novel regulatory gene of BABA-induced priming, *IMPAIRED IN BABA-INDUCED IMMUNITY1* (*IBI1*), which encodes an aspartyl-tRNA synthetase. In the absence of BABA, *IBI1* is transiently induced during pathogen infection. Moreover, trans-genetic over-expression of *IBI1* boosts disease resistance. Hence, IBI1 has a naturally occurring function in the plant’s innate immune system. Since priming is a cost-efficient defence strategy under high disease pressure, we considered the possibility that selected Arabidopsis accessions from hostile environments have evolved a constitutively primed immune system. We found that various Arabidopsis accessions display enhanced responsiveness to either pathogen-associated molecular patterns (PAMPs), or the defence hormone salicylic acid (SA). Genetic analysis of this natural variation identified different loci regulating responsiveness of PAMP- and SA-induced defence. Hence, priming of defence can be fixed genetically as an “innate” trait. However, most plants live in variably hostile environments, in which they would benefit from long-lasting priming that is reversible during intervening periods of low disease pressure. We, therefore, explored the hypothesis that priming is under epigenetic control, which would allow for long-lasting but reversible disease resistance. Recent evidence from our lab indicates that progeny from diseased Arabidopsis is indeed epigenetically primed to resist disease. All things considered, we conclude that the plant “innate” immune system is highly adaptive at different time scales, ranging from transiently expressed priming responses to much longer lasting adjustments that are based on (epi-)genetic modifications of the plant genome.

The genetically enriched (E)-β-ocimene in transgenic plants functions in indirect defenses in the both transgenic plants themselves and neighboring plants

Presenting author: **Gen-ichiro Arimura** (garimura@gcoe.biol.sci.kyoto-u.ac.jp)
Authors: Muroi A[1,2], Shimoda T[3], Nishihara M[4], Ozawa R[1], Takabayashi J and Arimura G[1,2]
[1]Department of Plant Molecular Biology, University of Lausanne, Switzerland
[1] Center for Ecological Research, Kyoto University, Otsu, Japan
[2]Global COE Program: Evolution and Biodiversity, Graduate School of Science, Kyoto University, Kyoto, Japan
[3]National Agricultural Research Center, Tsukuba, Japan
[4]Iwate Biotechnology Research Center, Kitakami, Japan

A blend of volatile organic compounds (VOCs) emitted from plants induced by herbivory enables the priming of defensive responses in neighboring plants. These effects may provide insights useful for pest control achieved with transgenic-plant-emitted volatiles. We therefore investigated, under both laboratory and greenhouse conditions, the priming of defense responses in plants (lima bean and corn) by exposing them to transgenic-plant-volatiles (VOCos) including (*E*)-β-ocimene, emitted from transgenic tobacco plants (NtOS2) that constitutively overepressing (*E*)-β-ocimene synthase. When lima bean plants that had previously been placed downwind of NtOS2 in an open-flow tunnel were infested by spider mites, they were more defensive to spider mites and more attractive to predatory mites (*Phytoseiulus persimilis*), in comparison to the infested plants that had been placed downwind of wild-type tobacco plants. This was similarly observed when the NtOS2-downwind maize plants were infested with *Mythimna separata* larvae, resulting in reduced larval growth and greater attraction of parasitic wasps. In a greenhouse experiment, we also found that lima bean plants (VOCos-receiver plants) placed near NtOS2 were more attractive when damaged by spider mites, in comparison to the infested plants that had been placed near the wild-type plants. More intriguingly, VOCs emitted from infested VOCos-receiver plants affected their conspecific neighboring plants to prime indirect defenses in response to herbivory.

We also investigated the effects abilities of transgenic wishbone flower plants (*Torenia hybrida*), emitting (*E*)-β-ocimene, on theto attraction of *P. persimilis*. Theis impact of (*E*)-β-ocimene was assessed in the natural VOC blends from the transgenic plants infested with spider mites, or with floral volatiles. *P. persimilis* were attracted to natural VOC blends from infested wild-type plants of *T. hybrida*. (*E*)-β-Ocimene stimulated theto ability to attract *P. persimilis* only when the trans-volatile was emitted together with the VOCs from the infested *T. hybrida* plants, in comparison to the attraction by infested wild-type plants. Intriguingly, floral volatiles masked the enhanced attractive effect of transformatnts.

Altogether, those data suggest that the genetically enriched (*E*)-β-ocimene can enhance the ability to prime indirect defenses in neighboring plants by attracting predators and cause indirect defenses in the transgenic plants only when added to an active VOC blend.

The role of root-specific MYB72 transcription factor during rhizobacteria-induced systemic resistance in *Arabidopsis*

Presenting author: **Christos Zamioudis** (C.Zamioudis@uu.nl)

Authors: Zamioudis C, Doornbos R, van der Ent S, Bakker PAHM, Pieterse CMJ

Plant-Microbe Interactions, Institute of Environmental Biology, Utrecht University, P.O. Box 800.56, 3508 TB Utrecht, The Netherlands

Root colonization by selected strains of non-pathogenic rhizobacteria triggers an induced systemic resistance (ISR) in diverse plant species that is effective against a broad spectrum of pathogens and even insects. In *Arabidopsis*, a transcriptomics-based approach identified the root-specific transcription factor MYB72 as an important component for the establishment of ISR. *MYB72* is locally induced upon root colonization by *Pseudomonas fluorescens* WCS417r and T-DNA mutants disrupted in *MYB72* abolished in their ability to generate ISR against a broad range of pathogens.

A survey in the *Arabidopsis* transcriptome using Genevestigator data revealed that *MYB72* expression is specifically induced under iron limited conditions. Here, we report that rhizobacteria capable of triggering ISR in *Arabidopsis*, are also able to upregulate iron deficiency mechanisms locally in the roots. We further demonstrate that WCS417r-induced expression of *MYB72* is depended on FIT1, the central regulator of iron acquisition in the roots. Constitutive high-level expression of *FIT1* is not sufficient to induce *MYB72* expression. However, overexpression of FIT1 together with either the bHLH38 or bHLH39 transcription factor converted the expression of *MYB72* to constitutive, indicating that the transcriptional regulation of *MYB72* during ISR is similar to that of the iron uptake genes *FRO2* and *IRT1*.

MYB72 is predominantly expressed in the vascular bundle; however, upon colonization by WCS417r, it is expressed in the epidermal and cortical cells. Microarray analysis further identified a number of WCS417r-induced genes that are regulated in a MYB72-dependent manner. These include the beta-glucosidase *BGLU42*, the cytochrome P450 monooxygenase *CYP71B5*, the oligopeptide transporter *NTR1.8* and a gene of unknown function. Remarkably, a cluster of defense-related genes showed increased expression in the *myb72* mutant and compromised expression in the *MYB72* overexpression line, indicating that WCS417r may trigger *MYB72* expression in order to attenuate local immune responses and establish successful infections. Accordingly, active root colonization by WCS417r was found to be impaired in the *myb72* mutant.

Lectin receptor kinases as modulators of the Arabidopsis innate immunity response

Presenting author: **Laurent Zimmerli** (lauzim2@ntu.edu.tw)

Authors: Desclos-Theveniau M, Singh P, Radziejwoski A, Chien SC, Huang TY, and Zimmerli L

Department of Life Science and Institute of Plant Biology, National Taiwan University, Taipei 106, Taiwan.

Diseases caused by microbial pathogens significantly contribute to the overall loss in crop yield worldwide. In order to better understand plant resistance to deleterious pathogens, my laboratory uses the priming phenomenon as a tool to discover new genes involved in the Arabidopsis defense response. Lectin receptor kinases play important role in animal innate immunity, but their possible involvements in plant resistance to pathogen remain largely elusive. I will provide information on lectin receptor kinases that were found to modulate the Arabidopsis innate immunity, in particular stomatal immunity

Molecular aspects of defense priming

Presenting author: **Uwe Conrath** (uwe.conrath@bio3.rwth-aachen.de)
Authors: Conrath U[1], Beckers GJM[1], Jaskiewicz M[1,2], Zhang S[3], Peterhänsel C[2,4]
[1]Department of Plant Physiology, RWTH Aachen University, Aachen 52056 Germany
[2]Department of Botany, RWTH Aaachen University, Aachen, 52056 Germany
[3]Department of Biochemistry and Bond LIfe Sciences Center, University of Missouri, Columbia, Missouri, 65211, USA
[4]Department of Botany, Hannover University, Hannover, 30419, Germany

Upon perception of microbe-associated molecular patterns, recognition of pathogen-derived effectors, colonization by beneficial microbes, treatment with various compounds or after wounding, plants can be primed for more rapid and robust activation of defense. Although the phenomenon has been known for decades, molecular mechanisms of priming remained elusive. It has been hypothesized that cell priming involves accumulation of latent signaling components that are not used until challenge exposure to stress. However, the identity of such signaling components has remained unknown. We showed that in *Arabidopsis thaliana* priming is associated with accumulation of mRNA and inactive proteins of mitogen-activated protein kinases, MPK3 and MPK6. Upon challenge exposure to biotic or abiotic stress, more of these enzymes were activated in primed plants than in nonprimed plants. This quantitatively greater activation was linked to enhanced PAL and PR1 defense gene activation and development of induced immunity.

More recent work showed that priming of the Arabidopsis WRKY29 defense gene promoter is associated with tri- (H3K4me3) and dimethylation (H3K4me2) of histone H3 Lys 4 (H3K4), and acetylation of H3K9, H4K5, H4K8, and H4K12. These modifications are normally found on active genes but WRKY29 remains inactive. Similar findings were made for WRKY6 and WRKY53 and mutant analyses revealed a tight correlation between histone modifications and gene priming. Thus, specific chromatin marks normally associated with gene activity are induced during priming before actual activation of defense, suggesting a histone memory in induced plant immunity.

Beckers et al. 2009. Plant Cell 21:944-953
Jaskiewicz et al. 2011. EMBO reports 12:50-55.

Properties and structure of the plant immune signaling network

Presenting author: **Fumiaki Katagiri** (katagiri@umn.edu)

Authors: Kenichi Tsuda[1], Masanao Sato[1]*, Yungil Kim[2], Jane Glazebrook[1], Chad Myers[2] and Fumiaki Katagiri[1]

[1]Department of Plant Biology and

[2]Department of Computer Science and Engineering, Microbial and Plant Genomics Institute, University of Minnesota, MN, USA

* Current address: Okazaki Institute for Integrative Bioscience, National Institute for Basic Biology, Okazaki, Japan.

The plant immune signaling network is different from other plant signaling networks because pathogens not only initiate signaling events but also interfere with plant signaling. Microbial pathogens also evolve much faster than plants. Therefore, the plant immune signaling network must have properties that allow it to withstand perturbations from a wide variety of pathogens without heavily relying on evolutionary adaptation. Unnecessary immune responses carry negative impacts on plant fitness, further constraining possible network properties. Pattern-triggered immunity (PTI) and effector-triggered immunity (ETI) are two modes of plant immunity. PTI is initiated by recognizing molecular patterns common among related microbes, including pathogens and benign microbes. Pathogens well-adapted to a host plant deliver effectors into the plant cell that interfere with PTI signaling and negate PTI. Plants may have receptors that recognize some of the pathogen effectors and trigger ETI, resulting in immunity. We demonstrated that at least some cases of PTI and ETI extensively share the signaling machinery and that what distinguishes PTI and ETI is the way the common signaling network operates. There is synergy among signaling sectors in PTI and compensation in ETI. The latter explains the robustness of ETI. In ETI compensation does not result from simple redundancy among sectors. We found that negative regulatory relationships between different signaling sectors are very common. Such prevalent negative regulatory relationships suggest that only part of the signaling network is highly activated at a given time. This likely reduces negative impacts of immune responses. If the primary sectors are perturbed by effectors, some other sectors may be released from suppression and provide back-up immunity, resulting in robustness of immunity against network perturbations by pathogen effectors.

Next generation Systemic Acquired Resistance

Presenting author: **Estrella Luna-Diez** (estrella.luna-diez@rothamsted.ac.uk)

Authors: Luna E[1,2], Bruce T[1], Flors V[3], Roberts M[2] and Ton J[1,4]

[1]Rothamsted Research, Centre for Sustainable Pest and Disease Management, Harpenden, UK.
[2]University of Lancaster, Lancaster Environmental Centre, Lancaster, UK
[3]University of Jaume I, Department CAMN, Laboratory of Biochemistry and Plant Biotechnology, Castellon, Spain
[4]University of Sheffield, Department of Animal and Plant Sciences, Sheffield, UK.

Systemic acquired resistance (SAR) is a long-lasting plant immune response to pathogen infection. We have investigated whether SAR can be transmitted to the next generation following exposure to disease by *Pseudomonas syringae* pv. *tomato* DC3000 (*Pst*DC3000). Compared to progeny from mock-inoculated plants (M_1), progeny from *Pst*DC3000-stressed plants (S_1) expressed enhanced resistance against (hemi-)biotrophic pathogens and were primed to activate SA-inducible defence genes. This trans-generational SAR still occurred after an intervening generation under stress-free conditions. However, S_1 plants also displayed reduced responsiveness of JA-inducible defence genes, which was associated with an increased susceptibility to the necrotrophic fungus, *Alternaria brassicicola*. S_1 progeny from the *npr1-1* mutant failed to develop trans-generational SAR and signalling cross-talk, suggesting an important regulatory role of NPR1. Chromatin immuno-precipitation analysis revealed post-translational modifications in histone H3 at promoters of SA- and JA-inducible genes between M_1 and S_1 progeny from wild-type plants, but not between M_1 and S_1 progeny from *npr1-1*. To assess the transmission of SAR to following generations, we quantified resistance in M_1 and S_1 progeny from the *dmr1,dmr2,ctm3* mutant, which is affected in non-CpG DNA methylation. M1 and S1 progeny of this triple mutant expressed similarly enhanced levels of resistance in comparison to wild-type M_1 progeny, which was associated with a priming of the SA response. These results indicate that hypo-methylation at non-CpG DNA transmits SAR to following generations. Future research will aim to elucidate the epigenetic mechanisms by which DNA-hypo-methylation directs trans-generational SAR.

Discovery of WRKY transcription factors involved in activation of SA biosynthesis genes; a bioinformatics and molecular biology approach

Presenting author: **Marcel Van Verk** (m.c.vanverk@uu.nl)

Authors: Van Verk MC[1,2], Bol JF[1], Linthorst HJM[1]

[1]Institute of Biology, Department of Plant Cell Physiology, Leiden University, the Netherlands

[2]Plant Microbe Interactions, Department of Biology, Utrecht University, the Netherlands

In order to comprehend the mechanisms of induced plant defense, knowledge of the regulation of the biosynthesis of the defense hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) is essential. Potentially, many transcription factors could be involved, but identifying them is a difficult endeavour. Using 372 publicly available microarray data sets, a network was constructed in which Arabidopsis genes known to be involved in SA, JA and ET signaling pathways together with over 1400 transcription factors genes were assayed for co-expression. Many obtained connections between transcription factors and genes involved in the signaling pathways were previously reported in literature to be relevant for stress responses and that fit current models of stress gene regulation, indicating the high potential of our approach. In addition, the derived network suggested new candidate genes and associations that are potentially interesting for future research to further unravel their involvement in responses to stress. Two of those candidate genes, *WRKY28* and *WRKY46*, were identified as possible regulators of *ICS1* and *PBS3* that are involved in SA biosynthesis. Expression studies with *ICS1 promoter::*β*-glucuronidase (GUS)* genes in *Arabidopsis thaliana* protoplasts cotransfected with *35S::WRKY28* showed that over expression of WRKY28 resulted in a strong increase in GUS expression. Moreover, qRT-PCR analyses indicated that the endogenous *ICS1* and *PBS3* genes were highly expressed in protoplasts overexpressing WRKY28 or WRKY46, respectively. Electrophoretic mobility shift assays indentified potential WRKY28 binding sites in the ICS1 promoter, positioned -445 and -460 base pairs upstream of the transcription start site. Mutation of these sites in protoplast transactivation assays showed that these binding sites are functionally important for activation of the *ICS1* promoter. Chromatin immunoprecipitation assays with haemagglutinin-epitope-tagged WRKY28 showed that the region of the *ICS1* promoter containing the binding sites at -445 and -460 was highly enriched in the immunoprecipitated DNA. The results obtained here confirm results from our multiple microarray co-expression analyses indicating that WRKY28 and WRKY46 are transcriptional activators of *ICS1* and *PBS3*, respectively, and support this *in silico* screening as a powerful tool for identifying new components of stress signaling pathways.

New chromatographic approaches reveal an active role of glucosyl salicylates against *P. syringae* in priming mutants

Presenting author: **Victor Flors** (flors@uji.es)
Authors: Pastor V[1], Barrera C[2], Cerezo M[1], Flors V[1]
[1]Metabolic Integration and Cell Signaling Group. Plant Physiology Section. Department of CAMN. Universitat Jaume I. 12071. Castellón. Spain
[2]SCIC. Universitat Jaume I. 12071. Castellón. Spain

Most studies about plant-pathogen interactions over the past decade have followed molecular and genetic approaches. Analyses of phytohormones and other plant growth regulators have provided useful information to understand how plants can adapt and prepare their primary and secondary metabolism to resist pathogens after pre-exposure to resistance-inducing agents. However, many induced resistance phenomena are based on a sensitisation or “priming” of defence, which only becomes apparent after pathogen infection as a faster and stronger activation of pathogen-inducible defences. Consequently, studies on hormones with a direct regulatory role in defence activation cannot fully explain the metabolic adjustments of the primed defence state. In the present study a new LC-mass spectrometry technique based on the full scan of hormone precursor conjugates revealed that SA conjugates play a relevant role as active hormone reservoirs in the priming mutant *nrt2* during *P. syringae* infection.

Plant basal resistance: genetics, biochemistry, and impacts on plant-biotic interactions

Presenting author: **Shakoor Ahmad** (shakoor.ahmad@rothamsted.ac.uk)

Authors: Ahmad S[1,2], Veyrat N[3], Gordon-Weeks R[1], Neil A[1], Pieterse CMJ[2], Ton J[1,4]

[1]Centre for Sustainable Pest and Disease Management, Rothamsted Research, UK

[2]Institute of Environmental Biology, Utrecht University, The Netherlands

[3]FARCE, University of Neuchâtel, Neuchâtel, Switzerland

[4]Department of Animal and Plant Sciences, University of Sheffield, Sheffield, UK

1

Basal resistance depends on a wide range of inducible defences that become active upon pathogen/insect attack. We have examined different aspects of basal defence in Arabidopsis and maize. It is commonly assumed that the speed and intensity of these inducible defences determines the effectiveness of basal resistance. To examine this further, we explored natural variation among Arabidopsis accession in defence responsiveness to pathogen-associated molecular patterns (PAMPs) and the defence hormone salicylic acid (SA). Quantitative trait loci (QTL) analysis of this natural variation identified loci regulating the sensitivity of these inducible defences. One QTL controlling SA responsiveness was found to contribute to basal resistance against *Pseudomonas syringae* pv. *tomato*. Next, we investigated the contribution of benzoxazinoids (BXs) in basal resistance of maize, using maize *bx1* mutant lines impaired in the first step of BX biosynthesis. Compared to wild-type lines, *bx1* lines displayed reduced penetration resistance against aphids and fungus. Furthermore, infestation of wild-type plants by aphids and fungi stimulated the conversion of DIMBOA-glucoside into HDMBOA-glucoside and DIMBOA, which was most pronounced in the apoplast of challenged tissues and preceded tissue damage or symptom development. Upon further investigation of wild-type and *bx1* mutant lines, we observed significantly reduced callose deposition in *bx1* plants after PAMP treatment. Furthermore, DIMBOA infiltration of the apoplast mimicked PAMP-induced callose. Hence, DIMBOA acts as a regulatory signal in aboveground cell wall defence of maize. BXs have also been reported to act as allelopathic signals in the rhizosphere. Analysis of root exudates revealed that DIMBOA is the dominant BX in root exudates of maize. To investigate the impact of BXs on plant-beneficial rhizobacteria, we monitored the impact of BXs on root colonisation by GFP-expressing *Pseudomonas putida* KT2440. Wild-type plants allowed more bacterial colonization than *bx1* plants, suggesting that BXs are involved in recruitment of beneficial rhizobacteria.

Photodynamic dyes may systemically reduce cucumber scab severity

Presenting author: **Andrey A. Aver'yanov** (aaveryanov@post.ru)
Authors: Aver'yanov A[1], Lapikova V[1], Pasechnik T[1], Baker CJ[2]
[1]Research Institute of Phytopathology, B. Vyazemy, Moscow region, Russia
[2]Agricultural Research Service, USDA, Beltsville, MD, the U.S.A.

2

The establishment of systemic acquired resistance by various chemical and biological agents applied to limited areas of the plant often involves the necrotization of the tissue that is in direct contact with the inducing agent. The cell death mechanism often involves overproduction of reactive oxygen species (ROS). Thus, factors capable of ROS-dependent local necrotization are of interest as potential plant resistance activators. Among such cytotoxic agents, photosensitizers, for example, photodynamic dyes deserve attention. Relatively little is known about their ability to control plant diseases.

The aim of this work was to test the ability of the photodynamic dyes (bengal rose, toluidine blue and methylene blue) to suppress systemically cucurbit scab caused by the fungus *Cladosporium cucmerinum* on cucumber.

It was found that these substances applied to the first true leaf at 0.5 to 200 μM caused restricted necrosis (scalds) on this leaf. Challenge inoculation of the second leaf (which emerged later) of these plants with conidial suspensions led to weaker disease severity in comparison with water-treated controls. The number and size of scald areas as well as suppression of the disease symptoms increased with dye concentration over a certain range. Both local and systemic effects were less pronounced on plants exposed to lower light doses for the first day after dye application.

The germination of fungal spores *in vitro* was strongly suppressed, in a light-dependent manner, by dyes at concentrations effective for systemic disease reduction. This direct fungitoxicity scarcely contributed to systemic effects of dyes but may alter the fungus upon direct contact under field conditions.

We suggest that photogeneration of singlet oxygen and other ROS by the dyes caused a local death of leaf cells, which subsequently produced mobile signals inducing disease resistance in distant parts of the plant. In direct contact with fungal cells, the dyes may cause their death due to oxidative damage. In our opinion, the photodynamic effects of these substances, both direct and systemic, offer a base for novel plant protectors.

The work was supported by the grant 4071p of ARS USDA through the mediation of the International Science and Technology Center.

Diversity in the effect of an extract from *Fallopia sachalinensis* on isolates of cucurbit powdery mildews grown on melon

Presenting author: **Marc Bardin** (marc.bardin@avignon.inra.fr)

Authors: Bardin M[1], Schmitt A[2], Nicot PC[1]

[1]INRA, Unité de Pathologie Végétale, B.P. 94, 84140 Montfavet, France

[2]Julius Kühn Institute, Institut für biologischen Pflanzenschutz, Darmstadt, Germany

3

Powdery mildew caused by *Podosphaera xanthii* and *Golovinomyces cichoracearum* is one of the principal diseases on cucurbit crops in temperate climates. In order to control this disease, various biological methods, including induced resistance by the use of extracts from *Fallopia sachalinensis*, have been identified.

This study was conducted to characterise the diversity of susceptibility to this plant extract among 52 isolates of *P. xanthii* and 5 isolates of *G. cichoracearum* collected from various cucurbit species in different production areas. To this end, disks excised from melon leaves were soaked in a preparation of *F. sachalinensis* extract (1% W/V) or in a control solution, inoculated with fresh conidia of powdery mildew 24 hours after treatment and placed in a growth chamber at 21°C. Ten days after inoculation, symptoms were rated individually for each leaf disk and were classified into 10 categories from 0 (no detectable fungal growth) to 9 (entire disk covered with heavy sporulation) based on a visual estimation of the leaf area infested by powdery mildew. Additionally, spore production on the leaf disks was assessed. The protective effect of the plant extract was estimated as the reduction in mildew severity on treated plants relative to that on the untreated control plants.

The plant extract significantly decreased the severity of disease for all the powdery mildew isolates tested, suggesting the absence of a high level of resistance to the effect of this product. On average for isolates of *P. xanthii*, spore production on the leaf disks was reduced five fold in presence of the plant extract relative to the untreated control. However, the extent of the reduction in spore production varied widely among isolates. Possible implications of these findings will be discussed.

Plant DAMPs and MAMPs operate similar conserved signalling pathways resulting in overlapping but also distinct defense responses

Presenting author: **Sebastian Bartels** (sebastian.bartels@unibas.ch)

Authors: Bartels S, Schulze B, Klauser D, Flury P, Böni R, Boller T

Botanisches Institut, University Basel, Basel, Switzerland

4

The innate immune response of animals and plants is triggered by the detection of exogenous and endogenous molecular patterns which are frequently linked to the situation of danger. In plants, the uncovering of the underlying molecular machinery has made considerable progress especially regarding the detection of exogenous MAMPs (microbe associated molecular patterns) and the subsequent signalling and response events. In contrast, the perception and functions of endogenous danger signals (DAMPs = damage associated molecular patterns) is to this day only sketchily understood.

Recently, the identification of peptidic DAMPs (AtPEPs) and their matching receptors (PEPR1 and PEPR2) enabled a closer investigation of DAMP-triggered innate immune responses in plants. It was shown that the perception of AtPEPs elicit responses intimately linked to defense including medium alkalinization, reactive oxygene species and ethylene production and the induction of several marker genes. Moreover pretreatment of Arabidopsis plants with AtPEPs led to an enhanced resistance against *Pseudomonas syringae* DC3000. Intriguingly these responses and the fact that PEPR1 and PEPR2 belong to the same class of receptors (LRR-RLKs) like the MAMP receptors FLS2 and EFR indicate that the MAMP and DAMP perception system shares downstream signalling pathways and regulatory elements.

Here we show our recent advances in the analysis of AtPEP perception and PEPR-triggered signalling. We found additional parallels between the MAMP and DAMP perception system like the interaction and phosphorylation of PEPR1 and BAK1, and the activation of the MAP kinases MPK3 and MPK6 upon flg22 as well as AtPEP detection. Moreover expression of PEPR1 and PEPR2 in the AtPEP insensitive plant *Nicotiana benthamiana* provided full responsiveness to AtPEPs indicating conserved downstream signalling pathways.

Beside the apparent parallels we also identified properties of AtPEPs distinct from the ones of classical MAMPs. Pretreatment of *Arabidopsis* plants with AtPEPs seems to improve the resistance to herbivores.

Brassinosteroids interact in beneficial bacteria mediated priming of plants for stress tolerance

Presenting author: **Sarosh Bejai** (Sarosh.Bejai@slu.se)

Authors: Fridborg I, Lagensjö J, Parra O, Meijer J and Bejai S

Department of Plant Biology and Forest Genetics, Biocenter, Swedish University of Agricultural Sciences, SLU, Uppsala, Sweden

5

Brassinosteroids (BRs) are steroidal hormones that are known to play crucial roles in plant growth and also promote tolerance to a range of abiotic and biotic stresses. Although a flood of information is available on the roles of BRs in plant development the mechanisms by which BRs control plant stress responses are not elucidated in detail. Pre-treatment of plants with beneficial bacteria (priming) has been an efficient strategy to protect plants against the onslaught of abiotic and biotic stress factors.

Transcriptomic analysis on *Brassica napus* leaf samples showed a number of BR responsive genes to be up-regulated upon priming by the biocontrol agent *Bacillus amyloliquefaciens.* To elucidate the role of BRs in Bacillus mediated priming of plants we performed more detailed functional analysis in *Arabidopsis thaliana.* Real-time PCR analysis of known BR responsive genes showed a differential transcriptional activation upon priming by Bacillus. Using a collection of Arabidopsis mutants devoid of BRs, we performed herbivory bioassays by the generalist larvae *Spodoptera littoralis* and specialist larvae *Plutella xylostella.* In order to understand the role of BRs in jasmonic acid (JA) signaling we employed virus induced gene silencing techniques to co-silence JA responsive genes in the BR mutants. Our results show a potential role for BRs during Bacillus mediated priming for enhanced plant growth and stress tolerance.

Regulation of barley (*Hordeum vulgare* L.) defense against the bird cherry-oat aphid (*Rhopalosiphum padi* L.)

Presenting author: **Lisa Beste** (lisa.beste@botan.su.se)

Authors: Lisa Beste[1], Sara Mehrabi[1], Inger Åhman[2], Lisbeth M. V. Jonsson[1]

[1]Department of Botany, Stockholm University, SE-106 91 Stockholm, Sweden
[2]Department of Plant Breeding and Biotechnology, Swedish University of Agricultural Sciences, Box 101, SE-230 53 Alnarp, Sweden

6

The bird cherry-oat aphid (*Rhopalosiphum padi* L.) is a phloem-feeding insect and a vector of plant viruses. High population densities of the aphid can cause substantial yield losses in cereal crops. The aim of our research is to identify and characterize resistance- and/or susceptibility genes in barley (*Hordeum vulgare* L.) and to identify factors, *e. g.* plant hormones, involved in the regulation of the plant defense mechanisms against aphids. An accession of wild barley (*H. vulgare* ssp. *spontaneum*) that exhibits antibiosis against *R. padi* has been crossed with a barley cultivar. Further back-crossings and selections have resulted in a number of barley lines in which we are looking for a correlation between the known levels of aphid resistance/-susceptibility and the expression level of candidate genes. A microarray analysis comparing the transcription profiles of barley genotypes possessing different levels of aphid resistance has revealed a number of genes that are differentially up- or down regulated after aphid infestation and a number of genes that are constitutively differentially expressed (Delp G et al., Mol. Genet. Genomics 281, 233, 2009). Sequence based annotation suggests a putative proteinase inhibitor and a putative Ser/Thr kinase among the gene products specifically induced in resistant barley lines. It has previously been suggested that plant defense against phloem-feeding insects is regulated by jasmonate (Walling LL, Plant Physiol. 146, 859, 2008). We report the constitutive gene expression of a selection of MeJA regulated genes in the barley breeding lines. In addition, to study the effect of individual candidate resistance- or susceptibility genes on aphid performance, selected cDNAs are presently being cloned to be expressed in sense and antisense direction in plants. To understand if the factors that bring on aphid resistance in our plants involve antixenotic and/or antibiotic mechanisms, we study both aphid settling and aphid reproduction on plants. New barley genotypes with enhanced endogenous resistance against aphids are part of a strategy in the breeding of crops to make it possible to reduce the use of pesticides.

Functional analysis of the RxLR5 effector of the Arabidopsis pathogen *Phytopthora brassicae*

Presenting author: **Lorelise Branciard** (lorelise.branciard@unifr.ch)

Authors: Branciard L, Stumpe M, Mauch F

Plant Biology, Dept. Of Biology, University of Fribourg

7

Oomycetes, including *Phytophthora* species, are known as notorious plant pathogens. We are interested in the weapons that *Phytophthora* uses to interfere with host immunity responses. To this end, we have identified a number of RxLR effectors in the Arabidopsis pathogen *Phytophthora brassicae*. Here, we focus on the functional analysis of RxLR5. Stable expression of RxLR5 in transgenic Arabidopsis led to a reduction of disease resistance against various pathogens suggesting that RxLR5 targets an important component of plant immunity. Gene expression profiling revealed an attenuated induction of a subset of *P. brassicae*-induced defense genes in RxLR5 expressing plants compared to wildtype. We have initiated yeast 2 hybrid screening to identify the putative target of RxLR5 at the molecular level. Initial results of this screening will be reported.

Metabolome and transcriptome analysis of the *Trichoderma* induced systemic resistance response to *Pseudomonas syringae* in *Arabidopsis thaliana*

Presenting author: **Yariv Brotman** (brotman@mpimp-golm.mpg.de)

Authors: Brotman Y[1], Lisec J[1], Chet I[2], Willmitzer L[1], Viterbo A[2]

[1]Max-Planck-Institut fur Molekulare Pflanzenphysiologie, Potsdam, Germany

[2]Department of Plant Pathology and Microbiology, The Hebrew University of Jerusalem, Rehovot 76100 Israel

8

In the present study we have assessed, by transcriptional and metabolic profiling, the systemic defense response induced by the beneficial fungus *Trichoderma asperelloides* T203 to the leaf pathogen *Pseudomonas syringae* pv tomato DC300 (Pst), in *Arabidopsis* plants.

We show that *Trichoderma* pretreated plants (+T203) showed significantly lower symptoms after infection with *Pst*. Using Agilent microarray in order to compare the expression pattern, in the leaf, of *Arabidopsis* plants 48 hours after colonization of the root with *Trichoderma* to control untreated plants, did not reveal substantial significant changes in genes expression.

In order to gain insight for the priming effect of *Trichoderma*, conferring resistance against *pst,* we selected genes, for qPCR analysis, that are known to be activated upon *Pseudomonas* challenge and / or involved in signaling resistance pathways to biotic and abiotic stress. Expressions of several genes were altered, For exp. the WRKY40 transcription factor, a negative regulator of defense response in *Arabidopsis* against *Pst*, was significantly reduced in *Pst* infected plants by *Trichoderma* root colonization.

At the metabolic level, while the bacterial infection causes a pronounced decrease in most of the compounds, especially sugars, *Trichoderma* root colonization has a milder effect on this class of compounds. Fungal root colonization induces significant changes in primary metabolites. (including amino acids, sugars and intermediates of the TCA cycle) which may reflect an increased energy supply required for the activation of plant defenses and growth promotion effects mediated by *Trichoderma* spp. We also observed that Fumaric and succinic acid were decreased upon *Pst* infection and a less extent in *Trichoderma* treated plants.

Aromatic amino acids are up-regulated in *Trichoderma* pre-treated plants and challenged by the bacteria. The shikimate pathway provides the basic building blocks for the synthesis of aromatic compounds required for different functions as: UV protection, electron transport, signaling, communication, plant defense, structural components and the wound response. Moreover phenylalanine is the precursor of the phenylpropanoid pathway with a key role in plant defense responses against *Pst*.

Molecular comparison of the ISR induced by different *Trichoderma* spp. against *Botrytis cinerea* infection in Arabidopsis and tomato

Presenting author: **Bruno Cammue** (bruno.cammue@biw.kuleuven.be)

Authors: De Cremer K, Yang Y, Vanhaecke M, De Coninck B, Cammue BPA, Mathys J

Centre of Microbial and Plant Genetics, Katholieke Universiteit Leuven, Kasteelpark Arenberg 20 , 3001 Leuven, Belgium

9

Based on a transcriptomic analysis, we previously characterized in detail the molecular mechanism underlying the systemic resistance (ISR) induced in *Arabidopsis thaliana* by *Trichoderma hamatum* T382 (T382) with protective effect against *Botrytis cinerea* infection. To increase the applicability of these findings, our results were extrapolated to tomato by means of a similar microarray analysis of the T382-induced ISR. The results of these experiments are being validated by qRT-PCR and mutant analysis. By comparing both microarray studies, we obtained a list of 42 genes that were induced in both Arabidopsis and tomato upon T382 treatment. In a next step, we evaluated which of these genes can be considered as general markers for the ISR that is induced by biocontrol fungi of the genus *Trichoderma*. To test whether different *Trichoderma* spp. use the same mechanism to induce ISR, we compared their effects on the expression of a selection of marker genes using qRT-PCR. We could indeed observe that different *Trichoderma* spp. that are able to establish a protective ISR effect against infection with *Botrytis cinerea*, induce the same marker genes while the expression of these selected genes remains unchanged upon treatment with *Trichoderma* spp. that do not induce such ISR.

Spatio-temporal study of two major PR-Proteins of grape berries during maturation, biotic and abiotic stress

Presenting author: **Steven Colas** (steven.colas@etudiant.univ-reims.fr)

Authors: Colas S, Crouzet J, Clément C, Baillieul F, Mazeyrat-Gourbeyre F, Monti-Dedieu L

Université de Reims Champagne-Ardenne, Laboratoire de Stress, Défenses et Reproduction des Plantes, URVVC-SE EA 2069, UFR Sciences Exactes et Naturelles, Moulin de la Housse, BP 1039, 51687 Reims Cedex 2, France

10

Pathogenesis-related proteins are important elements of the plant defense machinery. In grapevine (*Vitis.vinifera* L. cv. Pinot Noir) previous studies have shown that a chitinase (CHV5) and a thaumatin-like protein (TL) accumulate in berries during fruit maturation (Derckel *et al.* 1998; Davies and Robinson 2000) and have an antifungal effect against *Botrytis cinerea* (Derckel *et al.* 1998; Monteiro *et al*. 2003). However, *B. cinerea* is capable of growing on ripe berries. The aim of this work was to investigate firstly, the localization of these proteins in different tissues of berries during maturation and secondly, to study how their expression is affected by abiotic (UV-C) and biotic (*B. cinerea*) stresses. Localization of CHV5 and TL mRNAs/proteins was investigated by *in situ* hybridization and immunolocalization. Results show that during maturation without stress mRNAs/proteins of both were localized in the exocarp, and around all the vascular bundles of ripe berries. Following abiotic stress, after UV-C irradiation of berries at pre-veraison, *CHV5* and *TL* mRNAs accumulated in the exocarp and around all the vascular bundles. However, both proteins were localized in the exocarp and around the vascular bundles located in the mesocarp but not around those in the center of berries. In ripe berries from vineyards, CHV5 and TL mRNAs/proteins decreased during the infection by *B. cinerea*. Localization of both proteins in infected berries showed that proteins decreased around the site of infection, suggesting a degradation of both proteins by proteases secreted by the fungus (Have *et al*. 2004). Characterization of activities, properties, degradation of these two proteins produced by heterologous system is in progress. Finally, it is likely that these proteins, apart from being implicated in plant defense have also another function. To answer this question, transformation of grapevine plants, over- and underexpressing both genes are under progress.

References

Davies C and Robinson SP (2000): Differential screening indicates a dramatic change in mRNA profiles during grape berry ripening. Cloning and characterization of cDNAs encoding putative cell wall and stress response proteins. Plant Physiology 122, 803-812.

Derckel JP, Audran JC, Haye B, Lambert B, Legendre L (1998): Characterization, induction by wounding and salicylic acid, and activity against *Botrytis cinerea* of chitinases and β-1,3-glucanases of ripening grape berries. Physiology Plantarum 104, 56-64.

Have AT, Dekkers E, Kay J, Phylip LH, Van Kan JAK (2004): An aspartic proteinase gene family in the filamentous fungus *Botrytis cinerea* contains members with novel features. Microbiology 150, 2475-2489.

Monteiro S, Barakat M, Piçarra-Pereira MA, Teixeira AR, Ferreira RB (2003). Osmotin and thaumatin from grape: a putative general defense mechanism against pathogenic fungi. Phytopathology 93, 1505-1512.

Exploring natural genetic variation of plant responses to combinatorial stresses

Presenting author: **Silvia Coolen** (s.coolen@uu.nl)

Authors: Coolen S, Van Pelt JA, Van Wees SCM, Pieterse CMJ

Plant-Microbe Interactions, Faculty of Science, Utrecht University, The Netherlands

11

Biotic and abiotic stresses are major components of natural selection in the wild. In nature, plants have to cope with a wide range of biotic and abiotic stress conditions. As plants have co-evolved with an enormous variety of biotic and abiotic stresses, they harbour a fantastic reservoir of natural adaptive mechanisms to simultaneously cope with multiple stresses that until to date remained unknown or poorly understood. In order to gain new insights into how plants selectively adapt to the combined effect of pathogen infection, insect herbivory, and exposure to drought, we explore the resource of natural adaptive stress responses in *Arabidopsis thaliana* (*Arabidopsis*) to simultaneous interactions with multiple stresses. To this end, we analyze the effect of herbivory by caterpillars of *Pieris rapae* and drought stress on the level of resistance to the necrotrophic fungal pathogen *Botrytis cinerea* in the HapMap collection of 360 *Arabidopsis* accessions. This GWA-360 collection represents a wide variety of globally collected *Arabidopsis* plants that are genotyped for 250.000 single nucleotide polymorphisms (SNPs), which allows for genome-wide association mapping and cloning of genes of interest. We found that there is great natural variation in the effect of herbivory and drought stress on the level of *B. cinerea* resistance. Herbivory by *P. rapae* caterpillars or drought stress influenced the resistance against *B. cinerea* in many accessions, either positively or negatively. These data will be used for genome wide association mapping in order to identify novel genes that play a role in the capacity of plants to simultaneously adapt to multiple stresses, ultimately with the goal to utilize this knowledge to provide novel tools for sustainable agriculture and combinatorial stress resistance breeding and apply these tools to crop plants.

Did domestication reduce the attractiveness of maize for parasitoid wasps?

Presenting author: **Elvira S. De Lange** (elvira.delange@unine.ch)

Authors: De Lange ES, Gaudillat B, Farnier K, Turlings TCJ

Laboratory of Fundamental and Applied Research in Chemical Ecology, Institute of Biology, University of Neuchâtel, Switzerland

12

Artificial selection of crop plants for increased nutritional quality and yield might have a cost for other potentially useful traits, including resistance to herbivores [1]. Restoring defense traits in cultivated plants could be an effective way to combat pests. Besides direct defenses, such as the production of toxic compounds, plants may also indirectly protect themselves by emitting volatile organic compounds (VOCs) that attract the natural enemies of herbivores [2]. Parasitoid wasps are known to take advantage of these VOCs to localize hosts for their offspring. However, artificial selection of crops has reportedly led to the loss of these indirect defense signals [3].

The aim of this study was to identify possible differences in the attraction of organisms of the third trophic level by cultivated maize and its wild ancestor, teosinte. In a six-arm olfactometer, we compared the capacity of two teosinte subspecies (*Zea mays* ssp. *parviglumis* and *Z. mays* ssp. *mexicana*) and one cultivated maize line (*Z. mays* ssp. *mays* variety Delprim) to attract two species of parasitoid wasps (*Cotesia marginiventris* and *Campoletis sonorensis*). Two leaves of one plant of each genotype were artificially damaged and treated with caterpillar regurgitant to induce the emission of VOCs. The plants were then offered as odour sources in a three-choice situation to female wasps in an olfactometer.

Preliminary results show that the two teosintes were more attractive to the parasitoid wasp *C. marginiventris* than the cultivated maize, whereas *C. sonorensis* did not distinguish between the three plants. Interestingly, this wasp species tended to prefer the plants that emitted the highest total amount of VOCs. These results imply that two co-occurring species of parasitoid wasps exploit plant-provided signals differently. In addition, the results suggest that cultivated maize might have lost part of its ability to attract parasitoids. Previous studies showed that *C. marginiventris* is not attracted to the main maize VOCs, but rather to key compounds that are released in very small amounts [4]. Identification of these key compounds may contribute to the development of novel crop protection methods based on biological control.

[1] Rosenthal J.P. and Dirzo R. (1997). Evolutionary Ecology 11: 337-355.
[2] Heil M. (2008). New Phytologist 178: 41-61.
[3] Köllner T.G., Held M., Lenk C., Hiltpold I., Turlings T.C.J., Gershenzon J. and Degenhardt J. (2008). Plant Cell 20: 482-494.
[4] D'Alessandro M., Brunner V., Von Mérey G., Turlings T.C.J. (2009). Journal of Chemical Ecology 35: 999-1008.

Identification of putative protection markers in *Vitis vinifera* against *Botrytis cinerea*

Presenting author: **Betrand Delaunois** (bertrand.delaunois@univ-reims.fr)

Authors: Delaunois B[1,2], Colby T[3], Conreux A[1], Harzen A[3], Baillieul F[2], Clément C[2], Schmidt J[3], Jeandet P[1] and Cordelier S[2*]

[1]Université de Reims-Champagne Ardenne, Unité de Recherche «Vigne et Vins de Champagne – Stress et Environnement», Laboratoire d'Œnologie et de Chimie Appliquée, Faculté des Sciences, B.P. 1039, 51687 Reims cedex 02, France
[2]Université de Reims-Champagne Ardenne, Unité de Recherche «Vigne et Vins de Champagne – Stress et Environnement», Laboratoire de Stress, Défense et Reproduction des Plantes, B.P. 1039, 51687 Reims cedex 02, France
[3]Max-Planck-Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, D-50829 Köln, Germany

13

Grey mould caused by *Botrytis cinerea* infection is one of the main diseases that affect grapevine. The main solution to cope with this disease is the use of chemicals. Nevertheless chemical control of *B. cinerea* is impeded by the development of resistant strains. An alternative strategy to prevent diseases consists in stimulating plant defense mechanisms. To develop this technology, it appears essential to characterize biomarkers, which would enable to discriminate between defense stimulation and effective protection of grapevine against *B. cinerea*.

The aim of this study is to unravel protection biomarkers by two dimensional electrophoresis proteomic analysis comparing 'protective elicitors' with 'non protective elicitors' against *B. cinerea*. Differential analysis between those elicitors by 2D-PAGE allowed to characterize specific up- and down- regulated putative proteins (i) in whole leaf extract, namely, glutamine synthase, NAD-dependant epimerase/dehydratase and (ii) in the apoplastic fluid, namely, aspartic protease, isoflavone reductase like, glucanase, germine-like and serine-pyruvate aminotransferase. Further functional analyses of these proteins have to be performed to confirm their effective role in the protection of grapevine against *B. cinerea*.

The identification of protection biomarkers would improve the understanding of plant defense mechanisms against *B. cinerea* and allow to develop large scale screening tools for analysis of new elicitors regarding their protective effects against grey mould disease.

This work was supported by grants from the Région Champagne Ardenne and the Comité Interprofessionnel des Vins de Champagne.

Snapshot of the grapevine secretome using a proteomic approach

Presenting author: **Betrand Delaunois** (bertrand.delaunois@univ-reims.fr)

Authors: Delaunois B[1,2], Colby T[3], Conreux A[1], Harzen A[3], Baillieul F[2], Clément C[2], Schmidt J[3], Jeandet P[1] and Cordelier S[2*]

[1]Université de Reims-Champagne Ardenne, Unité de Recherche «Vigne et Vins de Champagne – Stress et Environnement», Laboratoire d'Œnologie et de Chimie Appliquée, Faculté des Sciences, B.P. 1039, 51687 Reims cedex 02, France
[2]Université de Reims-Champagne Ardenne, Unité de Recherche «Vigne et Vins de Champagne – Stress et Environnement», Laboratoire de Stress, Défense et Reproduction des Plantes, B.P. 1039, 51687 Reims cedex 02, France
[3]Max-Planck-Institute for Plant Breeding Research, Carl-von-Linné-Weg 10, D-50829 Köln, Germany

14

Apoplast is a continuous network in plants that creates an interface with the environment. After the cuticle, apoplast is the first barrier against pathogen attacks. In order to obtain an overview of the constitutive apoplastic proteome, a vacuum-infiltration-centrifugation method was optimized to collect the apoplastic fluid from grapevine leaves. Apoplastic protein profiles were compared to whole-leaf protein profiles by 2D-PAGE analysis. Tandem mass spectrometry analyses were used for protein identification. This approach allowed establishing a well-defined proteomic map of whole-leaf and apoplastic leaf proteins, with respectively 223 spots and 177 spots analyzed. To our knowledge, it is the first time that the apoplastic fluid is recovered from grapevine to characterize its protein content.

This study provides a comprehensive overview of the most abundant proteins present in grapevine apoplast. Protein function prediction allows us to conclude that the grapevine apoplast mainly contains stress-related proteins and proteins involved in cell wall metabolism. Moreover prediction tools revealed a high proportion of classical secreted proteins but also of non-classical secreted proteins namely Leaderless Secreted Proteins (LSP) in the apoplast. This approach provides a large number of candidate proteins involved in physiological functions of the apoplast under various stresses.

This work was supported by grants from the Région Champagne Ardenne and the Comité Interprofessionnel des Vins de Champagne.

Induction of resistance to *Botrytis cinerea* by ethylene is evolutionary conserved but ethylene inhibits vanillylnonanamide induced resistance

Presenting author: **José Díaz** (josefv@udc.es)

Authors: Díaz J, Carballeira R, Veloso J, García T

Departamento de Bioloxía Animal, Bioloxía Vexetal e Ecoloxía, Universidade da Coruña, Rua da Fraga, 10 E-15008 A Coruña, Spain

15

Ethylene is a plant hormone involved in resistance against pathogens, mainly necrotrophs as *Botrytis cinerea*. In the present paper we demonstrate the ability of ethylene to induce resistance against *Botrytis cinerea* and *Phytophthora capsici* in pepper. Jasmonic acid was also able to induce resistance against *B. cinerea*, but salicylic acid was not. In order to test if this ability to mediate resistance is evolutionary conserved, we tested ethylene in four plant species: *Phaseolus vulgaris* (Leguminosae), *Zinnia elegans* (Compositae), *Zea mays* (Poaceae, a monocotyledonous plant) and *Polypodium vulgare* (a fern). In all the cases ethylene caused resistance against *B. cinerea*. In pepper, ethylene induced the expression of *CASC1*, a sesquiterpene cyclase involved in phytoalexin biosynthesis, and chitinase activity. Both *CASC1* and chitinase could be related to the observed resistance.

Vanillylnonanamide is a pungent compound present in some pepper fruits. This compound was also able to induce resistance against *Botrytis* when exogenouly applied to pepper plants. The effect of vanillylnonanamide was also tested in the tomato ethylene signalling mutant *Never ripe*, where the induction worked, but in the wild type no induced resistance was observed. A similar result was obtained with salicylic acid: it only induced resistance to *Botrytis cinerea* in *Never ripe*, but not in the wild type. These results sugest a cross-talk between ethylene and salicylic acid in the vanillylnonanamide induced resistance. Non pungent capsaicinoid analogues, capsinoids, will also be tested in this scheme.

The present research was funded by Xunta de Galicia (Project 10MRU103009PR). JV holds a fellowship (María Barbeito Program) from Xunta de Galicia.

Elicitors from *Leptosphaeria maculans* inducing resistence in *Brassica napus* plants

Presenting author: **Kim Phuong Dinh** (dinhkimp@vscht.cz)

Authors: Dinh KP[1], Šašek VM[2], Burketová L[2], Valentová O[1]

[1]Department of Biochemistry and Microbiology, Institute of Chemical technology in Prague, Technická 1905/5, Prague 6- Dejvice, Czech Republic

[2]Laboratory of Pathological Plant Physiology, Institute of Experimental Botany AS CR, Na Karlovce 1a, 160 00 Prague 6- Dejvice, Czech Republic

16

Elicitors are important elements which activate defense mechamisms in plants after plant-pathogen interaction. *Leptosphaeria maculans* represents the most severe disease of oilseed rape causing blackleg stem canker. The aim of this study was to investigate the active substances secreted to the cultivation medium by *Leptosphaeria maculans* that are able to induce defense reresponse in oilseed rape plants against pathogens. The cultivation medium induced *PR-1* gene expression and enhanced resistance of plants to *Leptosphaeria maculans*. Active component was further characterized by cleavage with proteolytic enzymes trypsin and proteinase K and with glycosidases α-amylase and β-glucanase. The elicitor activity was eliminated by proteolytic cleavage while glycosidases had no effect. The activity was significantly lowered when the cultivation medium was heated to 80 °C. In addition, the dialysed and filtered medium was pre-fractionated by ion-exchange chromatography. Analysis of the most active fraction by mass spectrometry revealed mainly enzymes which can be involved in cell wall polysaccharides degradation.

This work was supported by Czech Science Foundation no 522/08/1581, Ministry of agriculture QH81201 and Ministry of Education MSM 6046137305.

Modulation of expression patterns of defense genes and stilbene productions in grapevine in response to biotrophic pathogen diversity (*Erysiphe necator* and *Plasmopara viticola*), after elicitation by benzothiadiazole

Presenting author: **Marie-Cécile Dufour** (dufour@bordeaux.inra.fr)

Authors: Dufour M-C[1], Bouscaut J[1], Lambert C[2], Cluzet S[2], Corio-Costet M-F[1]

[1]INRA, UMR Santé et Agroécologie du Vignoble (SAVE-1065), Institut des Sciences de la Vigne et du Vin (ISVV), BP 81, 71 rue Bourlaud, 33883 Villenave d'Ornon, France

[2]ISVV, GESVAB-EA3675, CS 50008, 33882 Villenave d'Ornon, France

coriocos@bordeaux.inra.fr

17

Benzothiadiazole (BTH) as salicylic acid analogue strengthened plant defense mechanisms against a broad spectrum of pathogens. Study was undertaken to assess the induction defense of BTH in grapevine (*Vitis vinifera*) in response to infection with different isolates of downy and powdery mildews (*Plasmopara viticola* and *Erysiphe necator*). In compatible interactions, without BTH treatment, amongst a set of 20 genes, some were up-regulated, but more than 57% in *P. viticola* infected-leaves (*Pv*-infected-leaves) and 90% in *E. necator* infected-leaves (*En*-infected leaves) of differentiated transcripts were down-regulated at 24 hpi indicating a possible manipulation of host response by pathogens. The follow-up of transcripts in *Pv*-infected-leaves or *En*-infected-leaves resulted in different widespread modulation of mRNA levels of grapevine, suggesting grapevine responses varied depending on inter and intra-species variability of pathogens and that specific up or down regulation of transcripts were involved in plant responses after BTH treatment either common or specific of pathogen species. After BTH treatment, grapevine inhibited pathogen development from 61 to 98 %, depending on pathogens, by triggering up-regulations of pathogenesis-related protein genes (PR-1, PR-2, PR-3, PR-10) in *Pv*-infected leaves whereas in *En*-infected leaves, PR-3, PR-10 and PR-6 transcripts were up-regulated. BTH led also to modulation in indole pathway transcripts in particular anthranilate synthase was down-regulated at 24 hpi in all infected-leaves then strongly up-regulated afterwards depending on the rate of pathogen development. To complete, polyphenols and stilbenes were quantified and our results demonstrated that only the pterostilbene was specifically accumulated in BTH treated leaves.

The significant over expression of PR-proteins and other defense involved genes (stilbene synthase, lipoxygenase, glutathion-S-transferase, and anthranilate synthase) could explain the high level of resistance against powdery and downy mildew in this susceptible cultivar and they are good candidates to be resistance markers inferred in some grapevine consequently could be helpful in the future development of chemical inducers in vineyards.

Induction of defense mechanisms in plant shoots may negatively affect root beneficial interactions with arbuscular mycorrhizal fungi

Presenting author: **Ivan M. Fernández** (ivan.fernandez@eez.csic.es)

Authors: Fernández I[1], De Román M[2], Wyatt T[1], Sahrawy M[3], Heil M[2, 4], Pozo MJ[1]

[1]Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín, CSIC, Profesor Albareda 1, 18008 Granada, Spain
[2]Department of Botany–Plant Ecology, University of Duisburg-Essen, Universitätsstrasse 5, 45117 Essen, Germany
[3]Department of Biochemistry and Molecular and Cellular Biology of Plants, Estación Experimental del Zaidín, CSIC, Profesor Albareda 1, 18008 Granada, Spain
[4]Departamento de Ingeniería Genética, CINVESTAV-Irapuato, km 9.6 Libramiento Norte, Carretera Irapuato-León, 36821 Irapuato, Guanajuato, México

18

Although most studies focus on shoot or root defense responses independently, evidences demonstrate they affect each other significantly. This fact has important implications from the practical point of view: On one hand, microbial communities in the soil may influence the resistance of aboveground tissues to pathogens as insects, as in the case of those inducing systemic resistance. Similarly, soil microorganisms may alter the efficiency of chemical elicitation of defences aboveground. On the other hand, elicitation of resistance mechanisms on the shoots may have undesirable side effects on beneficial communities in the rhizosphere. In legumes, chemical induction of resistance in the aerial compartment has been shown to spread to the roots and negatively affect the mutualistic interaction with rhizobia. In this study we analyzed the effect of the chemical induction of resistance in shoots of soybean plants on the arbuscular mycorrhizal (AM) symbiosis by using acibenzolar-S-methyl (ASM) as elicitor. The ASM application in plant shoots produced a moderate and transient decrease on AM root colonization. We analyzed different physiological and biochemical parameters (plant biomass, photosynthesis, sugar content and seed set, quantification of defense related enzymatic activities) to assess the mechanisms underlying such effect. The ASM application produced a moderate but significant activation of defense response in roots, which was modulated by AM, while no significant fitness costs were observed. The results indicate that the negative impact of defense elicitation in shoots on the AM interaction is related to changes in the defense status of the plant rather than to the costs associated to the induction of resistance.

Characterization of protease inhibitors in the grapevine-*Botrytis cinerea* interaction

Presenting author: **Clémentine Gérard** (clementine.gerard@etudiant.univ-reims.fr)

Authors: Gérard C, Crouzet J, Clément C, Mazeyrat-Gourbeyre F, Monti-Dedieu L, Baillieul F

Laboratoire de Stress, Défenses et Reproduction des Plantes, URVVC-SE EA 2069, Université de Reims Champagne-Ardenne, UFR Sciences Exactes et Naturelles, Moulin de la Housse, BP 1039, 51687 Reims Cedex 2, France

19

Pathogenesis-related (PR) proteins are important elements of the plant defence machinery against pathogens. Among these, PR-6 family includes proteases inhibitors (PIs) that are mostly studied for resistance to insects. Various studies suggest that the necrotrophic fungus *Botrytis cinerea,* the causal agent of gray mould, degrades defence proteins of grapevine by producing proteases (Manteau 2003; Marchal *et al,* 2006). Moreover, pepstatin, a chemical inhibitor of aspartic protease, was shown to reduce infection of carrot by *B. cinerea* (Movahedi & Heale, 1990). We hypothesize that the induction of PIs in grapevine may help the plant to counteract the infection by *B. cinerea*. The aim of this work is to identify PIs that could block fungal proteases and verify the impact of this inhibition on the ability of the fungus to degrade defence proteins of the plant. Two PIs, respectively part of the Potato Inhibitor I (VvPIN) and Kunitz Soybean Trypsin Inhibitor Family (VvKunitz16) were selected. Their expression pattern was studied by q-PCR according to various stresses including infection by *B. cinerea*. Preliminary results show that these two inhibitors are induced in berries by wounding, methyl jasmonate, an elicitor and during infection by the fungus but not by UV-C. Production of the two PIs is in progress and recombinants PI will be used to identify the fungal proteases interacting with them; to monitor their *in vitro* inhibitory activity against fungal proteases and their impact on the degradation of plant defence proteins. Finally, production of transgenic plants overexpressing PIs will allow checking their potential protector effect *in planta* against *B. cinerea*.

Manteau S. (2003). Thèse de doctorat : Etude des facteurs de virulence de Botrytis cinerea et des protéines de défense de la baie. Université de Reims Champagne-Ardenne.

Marchal R., Warchol M., Cilindre C., and Jeandet P. (2006). Evidence for Protein Degradation by *Botrytis cinerea* and Relationships with Alteration of Synthetic Wine Foaming Properties. J. Agric. Food Chem. 54 : 5157-5165.

Movahedi S. and Heale J. B. (1990) Purification and characterization of an aspartic proteinase secreted by Botrytis cinerea Pers ex. Pers in culture and in infected carrots. Physiological and Molecular Plant Pathology. Vol 36, Issue 4, 289-302.

Calcium as second messenger in wound-induced resistance

Presenting author: **Emna Hajri** (emna.beneloujaephajri@unifr.ch)
Authors: Hajri E, Binda M, Renevey V, L'Haridon F, Métraux JP
University of Fribourg, Department of Plant Biology, Switzerland

20

Wounded leaves of *Arabidopsis thaliana* produce ROS within minutes after wounding and are resistant to the pathogenic fungus *Botrytis cinerea* at a local level. This fast response of the plants to the wound is called *w*ound *i*nduced *r*esistance (WIR). However the molecular mechanisms of this response and the cascade signal between the wound and ROS production are still largely unknown. Calcium is a conserved second messenger and it is involved in many abiotic stress responses in plants (i.e. drought and salt stress). Furthermore, calcium pathways act very fast. For this reason, ROS staining with a specific fluorescent probe, microscopy and luminometry assay using plants expressing the calcium-sensitive protein Aequorin, were performed to investigate whether calcium was implicated in WIR and ROS production in *Arabidopsis thaliana*. The results of this study showed that leaves treated with calcium channels inhibitors (Verapamil and lanthanum) or calcium chelators (oxalate, EGTA, EDTA) are impaired in ROS production and are more susceptible to *Botrytis cinerea* after wounding. In addition, the intracellular measurement of calcium changes induced by the wound showed a cytosolic calcium influx few seconds after wounding. All the results obtained indicate calcium as a possible second messenger that plays an essential role in WIR.

Elicitra – integrated French network promoting the strategy of plant resistance induction by elicitors through research, training and development

Presenting author: **Sonia Hallier** (hallier@vegenov.com)

Authors: Hallier S[1] and Berthelot R[2]

[1]Vegenov-BBV, Penn ar Prat, F-29 250 St Pol de Léon

[2]ARVALIS-Institut du végétal, Station expérimentale, F-91 720 Boigneville

hallier@vegenov.com; r.berthelot@arvalisinstitutduvegetal.fr

21

Agriculture must face two challenging and apparently contradictory issues: become both competitive and sustainable. The current reduction plan of pesticide use, occurring throughout Europe, must therefore be accompanied by the development of efficient environmentally friendly methods in crop protection. Among them, the enhancement of plant defence mechanisms by elicitor treatments seems a very promising strategy and has become a major topic of current research.

Elicitra is a French network co-animated by ARVALIS-Institut du vegetal and Vegenov. Its main mission consists of understanding, developing and promoting strategies based on treatments of plants with elicitors. This network is dedicated to all crops. It includes partners from public and private research, technical institutes, universities, agricultural colleges, various actors of the crop industries and competitive clusters. By bringing together various partners with different skills ranging from field to lab and from research to training, the understanding and development of this alternative approach is to be accelerated. Elicitra is supported and financed by the French ministry of agriculture and fishing and was launched in 2010.

The objectives of this network are to:

- collect data and information of i) the expectations and needs of people from the plant industry; ii) elicitors, their characteristics and efficiency iii) the barriers to their development and use
- initiate discussion to improve our understanding of i) their modes of action; ii) the optimal conditions of their use under production conditions; iii) their impact on the environment and human health
- participate in the development of innovating tools directed to both professionals and scientists including a guide of standardized protocols for a reliable evaluation and good practice
- display information through a website and publications, and participate in the training of both professionals and students

Transcriptional regulation of defense gene expression in *Arabidopsis* by AtWRKY50

Presenting author: **Rana Muhammad Fraz Hussain** (r.m.f.hussain@biology.leidenuniv.nl)

Authors: Hussain RMF, Linthorst HJM

Department of Plant Cell Physiology, Institute of Biology, Leiden University, Sylviusweg 72, 2334 BE Leiden, the Netherlands

22

Salicylic acid is a plant hormone that mediates the induction of a defense response upon pathogen attack. Systemic acquired resistance (SAR) is tightly correlated with the expression of several classes of genes, collectively called SAR genes and its onset is associated with a substantial increase in endogenous salicylic acid (SA) levels, both near the site of infection and systemically. The *PR-1* gene is one of the genes induced during SAR. PR-1 proteins are widely used as markers for SAR. We are interested in the transcriptional activation of defense during SAR and use the Arabidopsis *PR-1* gene as a model gene in our studies. Previous work has indicated the importance of a region in the promoter of the *PR-1* gene for SA-induced expression. The region contains several potential binding sites for transcription factors that could be involved in the induced expression. The *AtPR-1* promoter binds the DNA binding domain (BD) of AtWRKY50 and 51; AtWRKY59 does not bind to the *PR-1* promoter. AtWRKY50BD specifically binds the LS10 element (GACTTTTC) previously found to be important for transcriptional activation of *PR-1*. AtWRKY50BD also specifically binds to an LS-10-like element in the *AtPR-2* promoter. Overexpression of AtWRKY50 in Arabidopsis protoplasts activates a co-transfected *PR-1::GUS* gene. Mutation of the LS10 element in the *PR-1* promoter reduced GUS expression. qRT-PCR on RNA from *35S::AtWRKY50*-transformed protoplasts showed that also the endogenous *PR-1* gene was expressed. Transformation with *AtWRKY51* or *59* genes did not result in *PR-1* over expression. In close proximity of the LS10 element two as-1 elements are present in the PR-1 promoter and one has been reported as a transcriptional enhancer element.As-1 elements have been shown to bind TGA transcription factors. We want to know if induced PR-1 expression involves interaction of TGA and WRKY factors. In protoplasts, concomitant presence of TGA2 or TGA5 and AtWRKY50 has a synergistic effect on PR-1-derived gene expression. Bimolecular fluorescence complementation(BiFC) assay showed that TGA2 and TGA5 interacts with AtWRKY50. Homozygous knockout lines were produced of *AtWRKY50,51 and 59*. These lines were crossed to obtain double homozygous plants. Also, transgenic (constitutive and inducible overexpression) plants of WRKY50, 51 and 59 are ready to be analyzed. These plants will be used for future analysis of these WRKYs' involvement in plant defense. In addition, the *PR-1* promoter will be used in yeast 1-hybrid assays to identify putative other WRKYs.

Phosphatidic acid produced by phospholipase D is necessary for salicylic acid induced gene expression in *Arabidopsis* seedlings

Presenting author: **Martin Janda** (mates.hawai@centrum.cz)

Authors: Janda M[1], Ježková L[1], Krutinová H[2], Šašek V[2], Burketová L[2], Martinec J[2], Novotná Z[1], Valentová O[1]

[1]Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, Czech Republic
[2]Institute of Experimental Botany AS CR, Prague, Czech Republic

23

The plant immune system is based on orchestration of complex events that results in an effective defence response to the pathogen attack. Signal transduction between cell surface and nucleus is mediated, among others, through the salicylic acid (SA) and jasmonic acid (JA) signalling pathways. Responses to pathogens also include activation of several phospholipid hydrolysing enzymes. One of the significant classes of these enzymes is phospholipase D (PLD) hydrolysing phospholipids to phosphatidic acid (PA) and a polar head. In *Arabidopsis* PLD is encoded by 12 genes divided into five distinct groups based on molecular structure and their biochemical properties. Expression profiling has shown that several PLD genes are induced by treatments with SA or JA.

In presented work, we studied involvement of PLD and PA in SA and JA signalling pathways. Production of PA through PLD activity is abolished in the presence of primary alcohols. Using qPCR, we investigated the effect of *n*-butanol on expression of SA and JA marker genes after treatment with chemical inducers of these pathways. Treatment of *Arabidopsis* seedlings with 0,1% n-butanol almost completely blocked the induction of SA marker gene *PR-1*. In the next step, we focused on identification of particular PLD genes participating in the SA mediated responses. Analysis of mutants impaired in single PLD genes has not identified a strong SA insensitive phenotype. We assume that PLD isoforms can act redundantly. Thus, we are crossing selected PLD mutants and using amiRNA we want to simultaneously silence all genes belonging to the PLDγ group.

Biocontrol of oilseed rape (*Brassica napus*) insect pests and pathogens, using *Bacillus amyloliquefaciens* bacteria and defence inducing chemicals

Presenting author: **Anna H. Johansson** (anna.h.johansson@slu.se)

Authors: Johansson A, Bejai S and Meijer J

Department of Plant Biology & Forest Genetics, Uppsala Biocenter, Swedish University of Agricultural Sciences, Box 7080, SE75007, Uppsala, Sweden

24

To support organic production of oilseed rape (*Brassica napus*) novel strategies need to be developed for sustainable crop protection to pathogens and insect pests. We are exploring the use of beneficial bacteria to prime plant defense. *Bacillus* species are interesting candidates being soil-living, facultative aerobe, endospore forming and often colonizing plant roots. *Bacillus* can produce many antibiotics and some strains are known to provide protection to plant pathogens.

An antifungal effect of different *Bacillus amyloliquefaciens* strains was previously demonstrated towards certain Brassica pathogens. Of the tested *Bacillus* strains, only UCMB-5036 showed a strong direct antagonistic effect *in vitro* against all the fungal species in plate tests. However, disease suppression on *Bacillus* inoculated plants showed another pattern towards the pathogens. This suggests that additional antifungal effects operate when the *Bacillus* strains are in contact with plants. Priming of plant defence as induced systemic resistance (ISR) is thus a likely mode of operation. Marker gene and mutant studies in Arabidopsis indeed showed that *B. amyloliquefaciens* disease suppression depends on jasmonic acid (JA) and ethylene indicative of ISR. We were therefore interested to study the potential of Bacillus strains or chemicals to prime defense against insects and pathogens on oilseed rape.

BABA (β-aminobutyric acid) is known to enhance defence in many plants against pathogens and some insect pests. BABA induced resistance is associated with priming for salicylic acid (SA) dependent defences against biotrophic pathogens. Benzothiadiazole (BTH) is a SA mimic used as inducer of SA controlled defences. The *B. amyloliquefaciens* strains, UCMB-5033, -5036, -5113 and FZB42 were also tested. The insects tested were Diamondback moth (*Plutella xylostella*) and flea beetle (Phyllotreta spp.), both specialist herbivores of Brassicas, and Cotton Leaf Worm (*Spodoptera littoralis),* a generalist feeder that can attack numerous plant species.

So far the study has been concerned mostly with the oilseed rape cv. Westar and tests were performed in controlled environment as well as under field conditions with experimental design both as choice and non-choice experiments. For Spodoptera and Plutella young larvae were used while flea beetles are caught or attracted by catch crops close to the test plants. The results did not show any significant effect of *Bacillus* treatment or chemical treatment against the tested insect species although a tendency of an improved plant defence was observed in certain cases. In contrast protection to fungal pathogens (*Verticillium longisporum and Botrytis cinerea*) could be observed. Accordingly *Bacillus amyloliquefaciens* mediated stimulation of different signalling pathways in plants does not automatically confer protection to insects. The study was supported by FORMAS and Helge Ax:son Johnssons fund.

ELK2, a MAPKKK that regulates the immune response of *Arabidopsis* to fungal pathogens

Presenting author: **Lucía Jordá** (lucia.jorda@upm.es)

Authors: Jordá L[1], Sopeña-Torres S[1], Sánchez-Rodríguez C[1], Escudero V[1] and Molina A[1]

Centro de Biotecnología y Genómica de Plantas (UPM-INIA), Departamento Biotecnología-UPM, Campus Montegancedo, M40 (Km. 38). Universidad Politécnica Madrid. 28223-Pozuelo de Alarcón, Madrid, SPAIN

25

Unlike animals, that have specialized cells to recognize foreign molecules to the organism, each plant cell has the molecular machinery needed to perform these functions of immunity. Efficient activation of plant defenses depends on the recognition of molecular patterns associated to microorganisms (MAMPs) to which the plant is exposed. The MAMPs are recognized by specific plant receptors (PRRs). This recognition leads to the activation of signaling cascades involving MAP kinases, which regulate the immune response. In a screening for mutants of *Arabidopsis thaliana* that were susceptible to fungal pathogens, *elk2* mutant was identified. This mutant is more susceptible than wild type plants to necrotrophic, biotrophic and vascular fungi, whereas its resistance to bacteria and oomycetes is not altered. This key regulator of resistance to plant pathogenic fungi has been cloned recently and found that encodes a MAP3K. Constitutive activation of this MAP3K confers resistance against a broad spectrum of fungal pathogens, confirming its role in the regulation of the immune response to fungi. ELK2 possibly regulates the signaling cascade activated after the specific recognition of fungal pathogens by one or more PRR, among which receptor-like kinases (RLKs) could be found. *elk2* mutant has an "*erecta-like*" developmental phenotype, suggesting that ELK2 may be involved in regulating the signaling pathway mediated by the RLK ERECTA. Advances in the characterization of the genetic and molecular mechanisms that determine the dual functionality of ELK2 in the regulation of plant development and innate immunity is presented in this work.

Up-regulation of genes encoding PR-proteins, lipoxygenases and a patatin-like lipase in pepper leaves inoculated with tobamoviruses

Presenting author: **Csilla Juhász** (juhasz.csilla@nki.hu)

Authors: Juhász C, Künstler A, Király L, Almási A, Tóbiás I, Gullner G

Plant Protection Institute of the Hungarian Academy of Sciences, Budapest, Hungary

26

Virus infections result in substantial alterations of gene expression patterns in infected plant tissues. Resistant plants respond to virus infections by a massive and rapid up-regulation of a wide variety of genes including those encoding the well-known pathogenesis-related (PR) proteins. Lipid-related defense reactions play also a substantial role in the virus resistance of plants. Patatin-like lipases can catalyze the hydrolyzation of different phospho- and galactolipids of various lipid membranes producing free polyunsaturated fatty acids (PUFAs). Lipoxygenases (LOXs) catalyze the peroxidation of PUFAs into fatty acid hydroperoxides, which can be further converted enzymatically to various bioactive oxylipins, including among others jasmonic acid, volatile C_6 compounds, divinyl ethers, and hydroxy fatty acids. To learn more about the mechanisms of virus resistance of pepper plants we have studied the expression of various defense-related genes by RT-PCR techniques in a pepper (*Capsicum annuum* L.) cultivar harbouring the L3 resistance gene. Mature leaves of this pepper cultivar were inoculated with two different *Tobamoviruses* in order to compare an incompatible and a compatible plant-virus interaction. Inoculation with *Obuda pepper virus* (ObPV) led to the appearance of hypersensitive necrotic lesions (incompatible interaction), while *Pepper mild mottle virus* (PMMoV) caused only very mild chlorotic symptoms (compatible interaction). Earlier we have shown that a divinyl ether synthase gene was massively up-regulated in ObPV-infected, resistant pepper leaves [1].

ObPV-inoculation led to a rapid and massive up-regulation of genes encoding the pepper pathogenesis-related (PR) proteins 4 and 10 in the infected leaves. In contrast, ObPV inoculation resulted only in a less intensive and slower induction in the expression of the gene encoding a basic PR-1 protein. The expression of all these PR-genes was only very slightly induced in PMMoV-inoculated leaves. No transcription was observed in mock-inoculated or in untreated control leaves. ObPV inoculation led also to a significant up-regulation of a patatin-like lipase (lipid acil hydrolase) gene as well as of those encoding several 9- and 13-LOX enzymes. In contrast, the expression of these genes increased in most cases to a lesser extent in PMMoV-inoculated, susceptible leaves and in mock-inoculated leaves. In summary, our results showed that the early and strong up-regulation of genes encoding PR-proteins, a lipase, and various 9- and 13-LOXs can contribute to the virus resistance of pepper plants.

1. Gullner G, Künstler A, Király L, Pogány M, Tóbiás I (2010) Physiol. Mol. Plant Pathol. 74, 387-393.

The financial support of the Hungarian Scientific Research Fund (OTKA K77641) is gratefully acknowledged.

Sulfate-induced resistance to *Tobacco mosaic virus* infection in tobacco is correlated with the induction of pathogenesis-related and antioxidant genes

Presenting author: **Lorant Király** (lkir@nki.hu)
Authors: Király L[1], Künstler A[1], Juhász C[1], Zechmann B[2], Müller M[2], Gullner G[1]
[1]Plant Protection Institute of the Hungarian Academy of Sciences, Budapest, Hungary
[2] University of Graz, Institute of Plant Sciences, Graz, Austria

27

A sufficient supply of sulfur can lead to sulfur induced resistance (SIR) or sulfur enhanced defense (SED), especially during fungal infections. However, the exact mechanisms behind SIR/SED and its function during viral infections remain unclear. We have shown earlier that tobacco plants (*Nicotiana tabacum* cv. Samsun *nn*) genetically susceptible to *Tobacco mosaic virus* (TMV) and grown with sufficient amounts of sulfate develop delayed and less severe systemic mosaic symptoms and display lower virus titers than plants grown on sulfate depleted medium [1]. The aim of the present study was to investigate if different degrees of sulfate supply can be correlated with changes in symptom development, virus contents and expression of pathogenesis-related (PR) genes in TMV-infected tobacco plants genetically resistant to the virus (cv. Samsun *NN*).

A sufficient sulfate supply (+S) in Samsun *NN* tobacco reduced the development of localized necrotic lesions and TMV accumulation during a hypersensitive response to about 50 % when compared to sulfate depleted (-S) plants. The occurrence of SIR/SED in +S plants was also signaled by a stronger induction of the defense marker genes *PR-1a*, *PR-1b*, *PRB-1b* and *PR-1c* during the first day after TMV-inoculation. A similar, early induction of antioxidant genes encoding a salicylic acid-binding catalase (*CATSAB*) and a Tau class glutathione S-transferase (*GSTau1*) was also observed.

In summary, our results demonstrate that in TMV-infected Samsun *NN* tobacco the occurrence of SIR/SED is correlated with a significant induction of PR-1 and antioxidant genes. Furthermore, the involvement of *CATSAB* points to the possible role of the defense signaling compound salicylic acid in this type of SIR/SED.

1. Höller, K., Király, L., Künstler, A., Müller, M., Gullner, G., Fattinger, M., Zechmann, B. 2010. Enhanced glutathione metabolism is correlated with sulfur induced resistance in *Tobacco mosaic virus*-infected genetically susceptible *Nicotiana tabacum* plants. Mol. Plant-Microbe Interact. 23, 1448-1459.

This research was supported by grants from the Hungarian Scientific Research Fund (OTKA K77641 and K84002).

The combined effect of soil drought and the two-spotted spider mite on maize antioxidant potential

Presenting author: **Malgorzata Kielkiewicz** (malgorzata_kielkiewicz@sggw.pl)

Authors: Czapla A[1], Kielkiewicz M[1], Zagdanska B[2]

[1]Department of Applied Entomology and
[2]Department of Biochemistry, Warsaw University of Life Sciences - SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland

28

In the present study, transgenic maize, resistant to target lepidopteran pests, and its non-transgenic counterpart were chosen to examine the involvement of key antioxidant enzymes scavenging reactive oxygen species in protection of the plant from oxidative damage triggered by the combination of biotic (non-target mite-pest) and abiotic (soil drought) stresses.

Six-week-old maize (expressing the *cry 1Ab* gene from *Bacillus thuringiensis* var. kurstaki, *Bt*) and non-*Bt* control plants at the ten-leaf-stage were subjected to:

(1) soil water deficit,

(2) two-spotted spider mite, TSSM (*Tetranychus urticae* Koch, Acari: Tetranychidae) attack (40 females/on the 7th leaf from the bottom) and

(3) two stresses simultaneously for 4 and 8 days.

Experiments were conducted in greenhouse conditions. After 4 and 8 days, leaves were detached from the control, mite-infested, soil drought-treated plants as well as those treated by both stresses simultaneously. Leaves were frozen in liquid nitrogen and stored at -80^{o}C until analyses. The activity of superoxide dismutase (SOD; E.C.1.15.1.1), catalase (CAT; E.C.1.11.1.6) and peroxidase (POX; E.C.1.11.1.7) in leaf samples was detected by in-gel activity staining methods.

It was found that compared to the control, in untreated leaves of *Bt* maize the activity of SOD isoforms was higher, whereas the activity of POX-2 isoform was lower and CAT-2 was the same. The activity of Mn-SODs in *Bt* maize more strongly increased than in non-*Bt* maize in response to 4-day stresses (single and combined). The TSSM was a stronger inducer of Mn-SODs activity than soil drought, however prolonged single and combined stresses decreased the activity of all forms of SOD. The activity of CAT-2 strongly decreased in *Bt* maize whereas CAT-2 activity increased in non-*Bt* stressed-maize in response to 4-days of single stress treatment. Only in the case of prolonged soil treatment was there an increase in CAT activity in both cultivars. Furthermore, soil drought was a more effective enhancer of POX activity than TSSM after 4 days in both cultivars, however the opposite was true for 8 day stresses.

In conclusion, short-term mite infestation, water deficit and the combination of both stresses triggered a differential and rather specific change of antioxidant protein activity in *Bt* and non-*Bt* maize cultivars.

Oxidative-stress related proteins in tomato leaves respond differently to the two-spotted spider mite feeding and soil drough

Presenting author: **Malgorzata Kielkiewicz** (malgorzata_kielkiewicz@sggw.pl)

Authors: Kielkiewicz M[1], Swiatek M[1], Godzina-Sawczuk M[1], Zagnanska B[2]

[1]Department of Applied Entomology and
[2]Department of Biochemistry, Warsaw University of Life Sciences - SGGW, Nowoursynowska 159, 02-776 Warsaw, Poland

29

One of the mechanisms involved in protecting the plant against the overproduction of reactive oxygen species is the increase of antioxidant activity. The aim of this study was to compare the engagement of enzymatic antioxidants in the tomato plant's response to two-spotted spider mites, TSSM (*Tetranychus urticae* Koch, Acari: Tetranychidae) attack and soil water shortage.

The tomato plants (6.5 week-old) of two cultivars: Motelle (*Mi-1.2,* no 255326) and Moneymaker (no 255325) (Centre de Recherches Agronomiques D'Avignon, INRA, France) were chosen as the plant material. The presence of the *Mi-1.2* gene product limits the development of nematods (*Meloidogyne incognita, M. javanica, M. arenaria*) and some insect herbivores (*Macrosiphum euphorbiae* Thomas, *Bemisia tabacci* Genn. and *Bactericerca cockerelli* Sulc). Plants of both cultivars grown under greenhouse conditions were divided into three groups, 6 per group: (1) the control – non-infested and water-treated; (2) infested by TSSM (50 females/leaflet; on the 3rd leaf from the bottom) and water-treated and (3) not water-treated and non-infested. All plants were underwent stress for 4 days. The mite-infested leaflets as well as leaflets from the 4th and 6th leaf of the infested and non-infested (the control) plants were detached and frozen until analyses. The same procedure was conducted on drought-stressed plants with one exception: the 3rd, 4th and 5th leaves were detached. The activity of superoxide dismutase (SOD; E.C.1.15.1.1), catalase (CAT; E.C.1.11.1.6) and peroxidase (POX; E.C.1.11.1.7) were analysed in-gel after electrophoresis of leaf protein extracts on polyacrylamide gel (PAGE).

It was found that the involvement of oxidative-stress related proteins is strongly dependent upon the type of stress (biotic *vs* abiotic), leaf position and tomato cultivars. Plants of the Moneymaker cultivar more strongly engage SODs and POXs in response to short-time drought than short-time TSSM feeding. However, the engagement of SODs and POXs is greater in response of the Motelle cultivar to mite-feeding than shortage of water in the soil. Generally, short-term stress (TSSM or soil drought) decreased the activity of CAT.

Inhibitors of antioxidant enzymes may systemically reduce cucumber scab severity

Presenting author: **Vera P. Lapikova** (aaveryanov@post.ru)

Authors: Lapikova V[1], Aver'yanov A[1], Pasechnik T[1], Baker CJ[2]

[1]Research Institute of Phytopathology, B. Vyazemy, Moscow region, Russia

[2]Agricultural Research Service, USDA, Beltsville, MD, the U.S.A.

30

Reactive oxygen species are recognized to participate in mechanisms of systemic acquired resistance. Many resistance inducers evoke oxidative bursts upon contact with plant tissues. This may be a consequence of both increased generation of reactive oxygen and decreased antioxidant activities.

In this work we attempted to systemically protect cucumber leaves from cucurbit scab (caused by *Cladosporium cucumerinum*) by local application of inhibitors of antioxidant enzymes.

The first true leaves were exposed to droplets of 0.25 μM to 10 мМ sodium diethyldithiocarbamate (DDC, inhibitor of Cu-Zn superoxide dismutase) or 0.1 to 1 мМ 3´-amino-1,2,4-triazole (AT, inhibitor of catalase). Water droplets were applied as a control. Second leaves (which emerged later) were inoculated with conidial suspensions.

At high concentrations, DDC was found to cause visual necrosis at the site of contact with the leaf. AT caused chlorosis of the whole plant. At lower concentrations (0.5- 1 μM DDC and 200-300 μM AT), both inhibitors did not alter plants visually but diminished disease severity. Germination of fungal spores *in vitro* was suppressed by both DDC and AT at concentrations effective for systemic reduction of the disease.

Therefore, inhibitors of antioxidant enzymes can systemically protect plants from the infective disease. Presumably, local suppression of antioxidant activities led to local oxidative burst, which in turn induced resistance in distant parts of the plant. Importantly, unlike some necrotizing inducers of resistance, the inhibitors of antioxidant enzymes tested were effective at doses that were not harmful to the plant. Under field conditions, the direct fungitoxic effects of these compounds may contribute to the disease control.

The work was supported by the grant 4071p of ARS USDA through the mediation of the International Science and Technology Center.

FMO1 and ALD1 mediate a common NPR1-dependent and SA-independent defence signal

Presenting author: **Andrea Lenk** (anle@life.ku.dk)

Authors: Lenk A, Pedersen C, Thordal-Christensen H

Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Thorvaldsensvej 40, 1871 Frederiksberg C, Denmark

31

In *Arabidopsis*, the lesion-mimic double mutant *syp121 syp122* exhibits multiple activated defence signalling pathways in the absence of pathogens. In a previous suppressor mutant screen, ALD1 (*AGD2-LIKE DEFENSE RESPONSE PROTEIN 1*) and FMO1 (*FLAVIN-DEPENDENT MONOOXYGENASE 1*) were discovered to play important roles for lesion formation and plant size retardation (Zhang *et al*., 2008). FMO1 and ALD1 have previously been identified to have an important role in pathogen defence. Although it is known that FMO proteins in general catalyze the transfer of hydroxyl groups to nucleophilic heteroatom-containing substrates such as sulfor, nitrogen, selenium, or iodine, the specific substrate and the product of FMO1 are yet unidentified. Furthermore, there are examples that FMOs can change the cellular redox state through the production of reactive oxygen species. ALD1 could likely be involved in lysine degradation as a lysine aminotransferase. However, as *ALD1* expression is low in *Arabidopsis*, lysine degradation may not be its main function.

In the present study, rosette leave size analysis of triple, quadruple and quintuple mutants in the *syp121 syp122* background suggests that ALD1 and FMO1 act on the same defence signalling pathway, which is independent of SA-signalling, but dependent on the SA-downstream target, NPR1.

Zhang Z, Lenk A, Andersson MX, Gjetting T, Pedersen C, Nielsen ME, Newman M-A, Hou B-H, Somerville SC, Thordal-Christensen H (2008). A lesion-mimic syntaxin double mutant in *Arabidopsis* reveals novel complexity of pathogen defense signalling. *Molecular Plant* 1, 510-527

Priming agent Hexanoic acid-enhaced resistance in mandarin Fortune against *Alternaria alternata*

Presenting author: **Eugenio Llorens** (ellorens@camn.uji.es)

Authors: Llorens E, Lapeña L, García-Agustín P*

Grupo de Bioquímica y Biotecnología Vegetal. Área Fisiología Vegetal. Departamento de Ciencias Agrarias y del Medio Natural ESTCE Universitat Jaume I

Campus Riu Sec. Castellón 12071, Spain

*E-mail: garciap@camn.uji.es

32

Previous investigations by our research group have demonstrated that hexanoic acid (priming agent, Hx) induces resistance in *Solanum lycopersicum* and *Arabidopsis thaliana* plants against *Botrytis cinerea* (Vicedo et al, 2009; Kravchuk et al, 2011). *Alternaria alternata* is the cause of abandonment of the hybrid mandarin Fortune (*C. tangerina* x *C. clementina*) due to Alternaria brown spot damage. In this work, induced resistance against citrus plants has been assessed. In order to study the mechanisms implied in IR-Hx against *A.alternata,* a histochemical analysis was carried out to assess the effect of reactive oxygen species ROS and callose deposition on production. We previously demonstrated that the number of infected leaves and the diameter of infection diminished when treating citrus plants with hexanoic acid 1 mM. Moreover, the Hx priming agent not only showed a beneficial effect in plant development, but also increased chlorophylls and proteins content, photosynthesis and transpiration rates and efficient water use. *A. alternata* infection quickly increased H_2O_2 and superoxide ions levels in the tissues. However, the Hx priming agent reduced the H_2O_2 level in leaves by at least four times on day 5 post-infection if compared with control plants. Likewise, superoxide lowered in treated plants. Furthermore, the priming agent seemed to considerably accumulate callose around the necrotic area, which could delay the development of infection. Hence, the obtained results support the idea that oxygen species ROS and callose could be involved in Hx-IR against *A. alternata* in citrus plants.

Vicedo et al 2009. MPMI vol 22,11 pages 1455-1465
Kravchuk et al, 2011. J. Plan Physiology. 168, pages 359-366

Are the strigolactones involved in plant defense responses?

Presenting author: **Juan A. Lopez-Raez** (juan.lopezraez@eez.csic.es)

Authors: Jiménez A, García JM, Pozo MJ, López-Ráez JA

Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín (CSIC), Granada, Spain

33

Strigolactones are a new class of plant hormones that were initially identified as signalling molecules playing a dual role in the rhizosphere as host detection signals for arbuscular mycorrhizal fungi and root parasitic plants (Bouwmeester *et al.*, 2007). Besides their importance in the rhizosphere, it has been recently shown that the strigolactones regulate above- and below-ground plant architecture (Gomez-Roldan *et al.*, 2008; Koltai, 2011; Ruyter-Spira *et al.*, 2011). Nowadays, novel roles for these plant hormones are being discovered, broadening our understanding of their functions in plant physiology. However, it is surprising that no relationship of the strigolactones with defense responses has been investigated so far.

In the present work, we analyzed the susceptibility of two strigolactone-deficient tomato mutants - *SlCCD7* and *SlCCD8* - to fungal pathogens. Two agronomical important pathogenic fungi, showing different life styles and affecting differentially the plant, were assayed. On one hand, we used *Fusarium oxysporum f.sp. lycopersici*, which is responsible of Fusarium wilt, a common vascular wilt fungal disease. On the other hand, the necrotrophic fungus *Botrytis cinerea* was also assayed. This fungus is responsible of the disease known as grey mould, causing considerable damage in tomato. The results will be discussed in relation to the possible role of the strigolactones as plant defense cues, as well as their interaction with other plant hormones such as jasmonic, salicylic and abscisic acid.

Bouwmeester HJ, Roux C, López-Ráez JA, Bécard G. (2007). Rhizosphere communication of plants, parasitic plants and AM fungi. Trends in Plant Science 12(5): 224-230.

Gomez-Roldan V, Fermas S, Brewer PB, Puech-Pagés V, Dun EA, Pillot JP, Letisse F, Matusova R, Danoun S, Portais JC, Bouwmeester H, Bécard G, Beveridge CA, Rameau C, Rochange SF. (2008). Strigolactone inhibition of shoot branching. Nature 455(7210): 189-194.

Koltai H. (2011). Strigolactones are regulators of root development. New Phytologist 190(3): 545-549.

Ruyter-Spira C, Kohlen W, Charnikhova T, van Zeijl A, van Bezouwen L, de Ruijter N, Cardoso C, López-Ráez JA, Matusova R, Bours R, Verstappen F, Bouwmeester H. (2011). Physiological effects of the synthetic strigolactone analog GR24 on root system architecture in Arabidopsis: another belowground role for strigolactones? Plant Physiology 155(2): 721-734.

Genetic separation of early and late responses to herbivory in Arabidopsis

Presenting author: **Massimo Maffei** (massimo.maffei@unito.it)

Authors: Maffei ME[1] and Maule A[2]

[1]Plant Physiology Unit, Dept. Plant Biology, Innovation Centre, University of Turin, Italy
[2]John Innes Centre, Norwich Research Park, Colney, Norwich NR4 7UH, UK

34

Upon herbivory by chewing insects, plants respond with a cascade of events that lead to the activation of defence mechanisms. These include perception of elicitors (or effectors of defense) by receptors, elevation of cytosolic calcium $[Ca^{2+}]_{cyt}$, plasma transmembrane (Vm) depolarization, ion efflux/influx, mitogen-activated protein kinase (MAPK) activation and protein phosphorylation, NADPH oxidase activation and reactive oxygen species (ROS) production, ethylene and jasmonate production, late defense response gene expression, and emission of volatile organic compounds (VOC). These events start locally at the feeding site but can spread systemically throughout the plant. Many pathogen effectors exhibit their activity across different plant species, suggesting that different plants possess related pathways for the activation of defense. Although the individual responses that comprise these pathways have been widely catalogued, the connections between them and their interdependence have been little studied.

Central to the success of these defences is the need for local and systemic communication between cells. For plant cells, surrounded by cell walls, cell continuity is achieved through the presence of plasmodesmata (PDs). These plasma-membrane-lined channels bridge the cell wall, provide symplastic continuity and provide soluble and membrane environments for the passage of small and some large molecules and potentially for electrical conduction. Following recent advances in our understanding of the molecular composition of PDs we tested the impact of mutations in specific PD-protein genes with respect to defense signaling in response to herbivory.Using Arabidopsis plants mutated for PD-located proteins (PDLP) we aimed to evaluate the involvement of PDs in defense against herbivory.

Critically, the experiments showed that the array of molecular responses to herbivory can be separated genetically from each other and from the overall defense response. Hence we show that changes in PD function affect the transmission of Vm signals upon herbivory and impact herbivore-dependent $[Ca^{2+}]_{cyt}$ signaling. The PDLP mutants also respond to herbivory with a burst of H_2O_2, which is clearly independent of Vm depolarization, and show reduced expression of the jasmonate pathway and altered expression of genes related to terpenoid synthesis. The latter effects correlate with the lack of herbivore response and herbivore-induced VOC emission. Our results reveal a somewhat unexpected role for PD in herbivory and indicate that we need to re-think the anticipated pathways for electrical and molecular communication that lead to defence against herbivores.

Analysis of the stimulation of the oxylipin pathway in tomatoes treated with the benefic rhizobacteria *Pseudomonas putida* BTP1

Presenting author: **Martin Mariutto** (martin.mariutto@doct.ulg.ac.be)

Authors: Martin Mariutto[1], Marie-Laure Fauconnier[2], Jean-Paul Wathelet[4], Marc Ongena[3], Philippe Thonart[3], Patrick du Jardin[2], Jacques Dommes[1]

[1]Laboratory of Plant Molecular Biology and Biotechnology, University of Liège
[2]Plant Biology Unit, Gembloux Agro-Biotech, University of Liège
[3]CWBI, Gembloux Agro-Biotech, University of Liège
[4]Laboratory of General and Organic Chemistry, Agro-Biotech, University of Liège

35

Two kinds of systemic resistances can be induced in plants by micro organisms: the systemic acquired resistance, which is generally induced by pathogens, and the induced systemic resistance (ISR), which is induced by Plant Growth Promoting Rhizobacteria (PGPR). A precedent study showed that the PGPR *Pseudomonas putida* BTP1 has the capacity to protect tomato plants against the pathogenic fungus *Botrytis cinerea* by inducing the ISR. We studied the effect of the bacteria in tomato before and after inoculation of the pathogen fungus, by analysing the stimulation of the defense oxylipin pathway. The activity of the first enzyme of the pathway, the lipoxygenase (LOX), was stimulated in treated plants before and two days after the infection, and was caused by the increase of the expression level of only two genes, LoxD and LoxF. One product of Lox, the 13-HPOT, was more abundant in treated plants under esterified forms before infection, but free forms, that did not show difference of accumulation between controls and treated before infection, accumulated more rapidly after pathogen inoculation in treated plants. Its reduced product, the 13-HOT, showed a similar profile. Downstream the Lox, the transcription level of allene oxide synthase, leading to the formation of jasmonic acid, and divinyl ether synthase, leading to the production of fungitoxic compounds, was similar: the transcription level was stimulated by the infection, but the increase was more important in control plants. The differential stimulation of the divinyl ether synthase was confirmed by the quantification of its products, the colneleic and colnelenic acids, which accumulated faster in control plants than in bacterised plants. Moreover, we did not monitor any accumulation of jasmonates in treated plants after infection. For another gene of the pathway, the hydroperoxide lyase (HL), leading to the production of defensive volatiles, no differences were detected between control and bacterized plants. This result was confirmed with the analysis of the enzymatic activity of HL which was not simulated by the infection nor by the treatment. These results strongly suggest that the resistance conferred by *P. putida* BTP1 is associated in tomato with the stimulation of the Lox pathway, and that the bacterial treatment leads to a faster and more adapted accumulation of fungitoxic compounds in tomato leaves after infection by *B. cinerea*, by stimulating the LOX and inhibiting the 13-HPOT-consuming enzymes.

Role of nitric oxide and plant hemoglobins in the host recognition of endosymbiotic, saprophytic and pathogenic fungi

Presenting author: **Ainhoa Martinez-Medina** (ammedina@cebas.csic.es)

Authors: Martinez-Medina A[1], Fernández I[1], García JM[1], Rodríguez-Serrano M[2], Sandalio LM[2], Romero-Puertas MC[2], Pozo MJ[1]

[1]Department of Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín (CSIC), Granada, Spain

[2]Department of Biochemistry, Cell and Molecular plant Biology, Estación Experimental del Zaidín (CSIC), Granada, Spain

36

NO has been identified as an important messenger in plant defence signalling against microbial pathogens (Romero-Puertas et al., 2004). Moreover, the involvement of NO during symbiotic interactions has been confirmed in legumes inoculated with their rhizobial partners, and plant hemoglobins are proposed to play a role in this process (Nagata et al., 2008). However, the function of NO and plant hemoglobins during plant-fungi interactions is largely unexplored, particularly regarding the arbuscular mycorrhizal (AM) symbiosis. In order to analyze a possible role of NO in the establishment of plant interactions with symbiotic and pathogenic fungi, we monitor the NO production in tomato roots during the early interaction with an obligate endosymbiont (the mutualistic AM fungus *Glomus intraradices*) a saprophyte (the beneficial fungus *Trichoderma harzianum*) and a pathogenic fungus (*Fusarium oxysporum*). A diferential pattern of NO accumulation was found in the three interactions. While a sustained increased in NO levels was found in the interaction with *G. mosseae*, only a week and transient increase was observed in the interaction with the saprophytic *T. harzianum*. In contrast, the pathogen produced an early and pronounced burst of NO in the host. In addition, the response to the pathogen was compared in susceptible and resistant tomato cultivars. Lastly, as non-symbiotic hemoglobins may act as NO scavengers and control various NO-regulated physiological processes of plants by modulating NO levels (Bustos-Sanmamed et al., 2010), the expression of the three known tomato hemoglobins have been analyzed during the different interactions under study. Consistent with the NO changes observed, a differential regulation of the hemoglobins was observed. Our results suggest a role for NO and plant hemoglobins in the host recognition of different microorganisms and the establishment of the interaction.

Romero-Puertas MC; Perazzolli M; Zago ED; Delledonne M. 2004. Cell. Microbiol. 6: 795-803.
Nagata M; Murakami E; Shimoda Y; Shimoda-Sasakura F; Kucho K; Suzuki A; Abe M; Higashi S; Uchiumi T. 2008. Mol. Plant Microbe Interact. 21:1175-1183.
Bustos-Sanmamed P; Tovar-Méndez A; Crespi M; Sato S; Tabata S; Becana M. 2010. New Phytol.189: 765-776.

Differential induction of resistance to leaf rust in wheat cultivars by root-colonizing bacteria

Presenting author: **Fabio Mascher** (fabio.mascher@acw.admin.ch)
Authors: Mascher F[1], Kellenberger S[1], Farzaneh M[2,4], Péchy-Tarr M[3], Keel C[3], Sharifi-Tehrani A[1,4]
[1]Agroscope Changins-Wädenswil, CH-1260 Nyon, Switzerland, e-mail: fabio.mascher@acw.admin.ch;
[2]Medicinal Plants and Drugs Research Institute, Shahid Beheshti University, Evin, Tehran, Iran, e-mail: mfarzane@alumni.ut.ac.ir
[3]Department of Fundamental Microbiology, University of Lausanne, CH-1015 Lausanne, Switzerland, e-mail: christoph.keel@unil.ch
[4]Department of Plant Protection, University of Tehran, Karaj, Iran, e-mail: asharifi@chamran.ut.ac.ir

37

Plants dispose of several types of resistance mechanisms to cope with pathogens. These resistance types are commonly classified in quantitative, non specific resistance and vertical, host specific resistance. The resistances response depends upon plant variety, pathogen virulence, environmental conditions, growth stage of the plant and interactions with other organisms such as disease suppressive micro-organisms. Several root associated bacteria do not only inhibit the growth of pathogens, but they also induce systemic resistance (ISR) in the plant hence conferring protection on leaves. Only little is known about the interactions between plant resistances and ISR inducing bacteria, in particular about the genetic basis governing this interaction. The present study explored the effects of biocontrol strains of Pseudomonas fluorescens on the resistance of wheat varieties against leaf rust disease caused by Puccinia triticina. The wheat cultivars included highly susceptible (Cimetta and Arina) and intermediate resistant (Zinal and Forno). While cultivars Cimetta and Arina dispose of resistance gene Lr12 that is compatible with the P. tritina strain used, cultivar Forno disposes of quantitative resistance provided by resistance gene Lr34. Resistance of Zinal is not complete, indicating the presence of quantitative resistances of unknown origin. Inoculation of roots with biocontrol pseudomonads reduced significantly the severity of symptoms on the leaves in all wheat cultivars. Interestingly, the degree of disease reduction was lowest in Forno but highest in Zinal. The results highlight that level between wheat and bacteria inducing ISR take place at the genotype level.

The NIMIN proteins have differential effects on the expression of *PR1* genes

Presenting author: **Ashir Masroor** (ashermasroor@hotmail.com)
Authors: Masroor A, Maier F, Pfitzner UM
Universität Hohenheim, Institut für Genetik, FG Allgemeine Virologie, D-70593 Stuttgart

38

NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1) is a regulator of salicylic acid (SA)-mediated expression of *PATHOGENESIS-RELATED (PR) PROTEIN1* genes (Cao et al., 1997; Ryals et al., 1997). Current evidence suggests that NPR1 is part of a transcription complex tethered to *activation sequence-1* (*as-1*)-like *cis*-acting elements in *PR1* gene promoters through TGA transcription factors, and that SA-dependent *PR1* gene expression is regulated by NIM1-INTERACTING (NIMIN) proteins. Arabidopsis contains four *NIMIN* genes which are regulated differentially. Whereas *NIMIN1* and *NIMIN2* are induced by SA, *NIMIN3* is expressed constitutively at a low level. Furthermore, the NIMIN proteins interact differentially with NPR1. NIMIN1, NIMIN1b and NIMIN2 bind to a conserved domain in the NPR1 C-terminus. In yeast two-hybrid analyses, interaction of these proteins with NPR1 is sensitive to SA (Maier et al., 2011). In contrast, NIMIN3 interacts with another region, and binding of NIMIN3 to NPR1 is not affected by SA (Maier et al., 2011). Interestingly, plant species outside of the family of *Brassicaceae*, e.g. tobacco, do not seem to contain *NIMIN1* and *NIMIN3* homologous genes (Zwicker et al., 2007). Rather, tobacco harbours various genes related to *NIMIN2* (Zwicker et al., 2007). In line with this, the Arabidopsis *NIMIN* genes have recently been classified as lineage-specific genes implying that they are fast evolving genes, and that the encoded proteins are important for environmental adaptation of the plant pathogen response (Donoghue et al., 2011; Zwicker et al., 2007; Maier et al., 2011). Together, these findings strongly suggest that the NIMIN proteins fulfill different functions during the SAR response. To understand the significance of diverse NIMIN proteins, we have developed a transient gene expression system to study the impact of NIMINs on *PR1* gene regulation. Transient expression of NIMIN1 suppresses the SA-mediated activation of *PR1* genes. This finding is consistent with previous results on stable overexpression of NIMIN1 in transgenic Arabidopsis plants (Weigel et al., 2005). The effects of various NIMIN proteins on *PR1* gene expression will be presented.

Cao *et al*. (1997) Cell 88, 57-63.
Donoghue *et al*. (2011) BMC Evolutionary Biology 11:47.
Maier *et al*. (2011) Mol. Plant Pathol. 12, 73-91.
Ryals *et al*. (1997) Plant Cell 9, 425-439.
Weigel *et al*. (2005) Plant Cell 17, 1279-1291.
Zwicker *et al*. (2007) Mol. Plant Pathol. 8, 385-400.

How long does induced resistance last?

Presenting author: **Livia Atauri Miranda** (livia.atauri@unine.ch)

Authors: Slaugther A, Miranda LA, Brigitte Mauch-Mani

University of Neuchâtel, Laboratory of molecular and cell biology, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

39

Plants throughout evolution have developed very sophisticated defense mechanisms against biotic and abiotic stress. These stresses influence plants in different ways, such as affecting their physiology or altering their resistance responses. Plant resistance can be enhanced by the use of different natural and synthetic components that will induce “priming”. A primed plant will respond faster and stronger when confronted with a biotic or abiotic stress. Interestingly, primed plants do not show major trade-offs in growth and seed production. The inheritance of priming is analyzed in this work. It presents that descendants of plants that had been exposed to stress showed an increased priming and resistance response when confronted with pathogens. We will present our results concerning Arabidopsis plants that have been either primed with β-amino-butyric acid (BABA) or with an avirulent isolate of the bacteria Pseudomonas syringae pv tomato (Pst). The primed state induced by BABA-or bacterial treatment of parent plants was transferred to the next generation. The descendants of primed plants showed a faster and higher accumulation of transcripts of defense-related genes and enhanced disease resistance upon challenge inoculation with a virulent isolate of Pst. In addition, the progeny of primed plants was also more resistant against the oomycete pathogen *Hyaloperonospora arabidopsidis*. When transgenerationally primed plants where subjected to an additional priming treatment, their descendants displayed an even stronger primed phenotype. Our results demonstrate that the primed state of stressed plants is transferred to their progeny and confers improved protection from pathogen attack as compared to the descendants of non-primed plants.

Barley genes needed for powdery mildew infection

Presenting author: **Sara M. Mørch** (saram@life.ku.dk)

Authors: Sara M. Mørch[1], Dale Godfrey[2], Patrick Schweizer[3], Hans Thordal-Christensen[1]

[1]Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Denmark
[2]Present address: School of Agriculture, Food & Wine, The University of Adelaide, Australia
[3]IPK-Gatersleben, Germany

40

This is a study of the interaction between the biotrophic pathogenic powdery mildew fungus (*Blumeria graminis* f.sp. *hordei*, *Bgh*) and its host barley. A successful interaction with the living plant cell depends on establishment of specialised organs called haustoria. These are formed inside the living host cells and are used to retrieve nutrients to support fungal growth and propagation. Identification of barley genes needed for the establishment of *Bgh* haustoria will be of great help in further understanding this intimate relationship and will have importance in future efforts to create resistant plants.

An EST library based on the interaction between barley and *Bgh*, has previously been created (Godfrey *et al.* 2010). This library consists of ~10,000 EST sequences from epidermal barley cells containing *Bgh* haustoria. Analysis of these has classified 3,416 of them as barley ESTs of which 178 represent novel barley genes. Some of these genes are expected to have importance for the establishment of haustoria. In order to identify which are important, single cell gene-silencing by RNA interference (RNAi) was performed. This was conducted by shooting gold particles coated with RNAi constructs into epidermal cells of barley leaves, followed by inoculation with *Bgh*. Susceptibility was assessed based on development of haustoria in the silenced cells.

For the preliminary screen, 88 genes were tested by RNAi and 19 gave interesting phenotypes. After five separate re-tests, it was confirmed that silencing of five of the 19 genes caused significant resistance to *Bgh*. The five barley genes all represent negative regulators of defence in barley. Future actions will be taken in order to shed light over the molecular functions of these five promising candidates.

Godfrey D, Böhlenius H, Pedersen C, Zhang Z, Emmersen J, Thordal-Christensen H (2010), Powdery mildew fungal effector candidates share N-terminal Y/F/WxC-motif, *BMC Genomics* 11: 317

Performance of *Bemisia tabaci* on tobacco plants over-expressing the phenylpropanoid-biosynthesis pathway is affected by a negative cross-talk between the salicylic acid and jasmonic acid defense signaling pathways

Presenting author: **Shai Morin** (morin@agri.huji.ac.il)

Authors: Morin S[1], Alon M[1], Elbaz M[1], Spitzer B[2], Moyal Ben Zvi M[2] and Vainstein A[2]

[1]Department of Entomology, The Hebrew University of Jerusalem, Rehovot 76100, Israel

[2]Robert H. Smith Institute of Plant Sciences and Genetics in Agriculture, The Hebrew University of Jerusalem, Rehovot 76100, Israel

41

Phenylpropanoid metabolism comprises a complex series of branching biochemical pathways that provide plants with thousands of compounds, which are widely used in the anti-microbial and anti-herbivore defense arsenal. Recently, we have produced tobacco (*Nicotiana tabacum)* plants that over-express the MYB transcription factor *Pap1* (Production of Anthocyanin Pigment 1), which activates the phenylpropanoid-biosynthesis pathway through transcriptional up-regulation of *Pal* (phenylalanine ammonia-lyase, coding the enzyme catalyzing the first committed step in the phenylpropanoid pathway). This transgenic manipulation allowed us to investigate the effect of high levels of phenylpropanoids / flavonoids on host selection, oviposition, development and survival of *Bemisia tabaci* (Hemiptera: Aleyrodidae), a phloem feeding generalist insect model.

Host selection and oviposition choice experiments showed that *B. tabaci* females (B biotype) preferred as hosts the wild type plants over the *Pap1*-transgenic plants. Surprisingly, however, percentage of egg hatching was significantly higher and nymphal development rate significantly faster on *Pap1*-transgenic plants than on wild type plants. Moreover, in no-choice experiments, significant higher number of individual adults survived long periods of feeding *Pap1*-transgenic plants than on wild type plants. In addition, significant higher number of eggs was oviposited on *Pap1*-transgenic plants than on wild type plants. Taken together, these findings indicated that over-expression of the phenylpropanoid-biosynthesis pathway made the over-expressing plants a more suitable host for *B. tabaci* than wild type plants.

Transcriptional and biochemical analyses of the wild type and *Pap1*-transgenic plant's salicylic acid (SA)- and jasmonic acid (JA)-dependent defense signaling pathways suggested that the differences in insects performance are likely to be related to a relative enhanced expression of the SA and JA defense pathways in the *Pap1*-transgenic and wild type plants, respectively.

As recent studies on *Arabidopsis thaliana* signaling mutants revealed that phloem-feeding insects performance and success depends on the suppression of the JA-signaled events, we suggest here that *B. tabaci* responses to *Pap1*-transgenic plants reflect a complicated counter balance between to two strong defense strategies: the production of toxic phenolic compounds and the jasmonate-regulated defenses. The possibility that transcriptional up-regulation of *Pal* can lead to suppression of JA-based defenses, although *Pal* is known to be positively regulated by JA, will be discussed.

Promoter analysis of pathogen inducible genes of grapevine

Presenting author: **Tina Moser** (tina.moser@jki.bund.de)
Authors: Moser T[1], Merz P[2], Bogs J[2] and Zyprian E[1]
[1]Julius-Kühn-Institut, Institut für Rebenzüchtung Geilweilerhof, 76833 Siebeldingen
[2]Dienstleistungszentrum ländlicher Raum Rheinpfalz, Studiengang Oenologie und Weinbau, 67435 Neustadt

42

Grapevine is one of the most important fruit species worldwide. In Europe only *Vitis vinifera ssp. vinifera* is cultivated. However, this species is susceptible to Powdery and Downy Mildew caused by *Erysiphe necator* and *Plasmopara viticola* which were introduced from North America in the 19th century. Since then it is necessary to treat the plants with high amounts of fungicides which causes high costs and is environmentally unfriendly. These measures can be reduced by breeding and use of resistant grapevines. Modern breeding is accelerated by the use of molecular markers. The understanding of the mechanism of pathogen defense could strongly improve the development of resistance correlated markers and support the combination of different mechanism of resistance.

In previous work candidate genes were described that are upregulated in resistant plants after the attack of *Erysiphe necator* (Welter et al, submitted). These candidate genes are PR5, PR10 and some transcription factors. In the actual project, the promoters of these genes were cloned from a resistant and a susceptible grapevine. The promoters were sequenced and analyzed *in silico*. The transcriptional regulation of the promoters will be analyzed in transient expression systems with the help of reporter genes. Furthermore we will functionally characterize the candidate genes.

Response of *Arabidopsis thaliana* wildtype *Landsberg erecta (Ler)* and mutant *hpl1* to *Pseudomonas syringae i*nfection

Presenting author: **Cynthia Nyambura Mugo** (cynthmugo@yahoo.ca)

Authors: Mugo CN[1], Scala A[2], Matsui K[1], Takabayashi J[3], Schuurink RC[2]

[1]Graduate School of Medicine (Agriculture), Yamaguchi University

[2]Department of Plant Physiology, University of Amsterdam

[3]Center for Ecological Research, Kyoto University

*Corresponding author: matsui@yamaguchi-u.ac.jp

43

P. syringae pv. tomato (Pto) DC3000 (DC3000) causes bacterial speck of tomato and is also a pathogen of the model plant *Arabidopsis thaliana*. DC3000 colonizes the intercellular spaces of aerial organs upon entry via stomata or wounds. In the apoplast DC3000 encounters metabolites such as γ amino butyric acid (GABA) which it utilizes as carbon and nitrogen source. Previously, it was found that growth of Δ*gabT*, a mutant lacking all the three *gabT* GABA transaminase genes was weakly reduced in *Ler* in comparison to the wild type strain DC3000 (Mirabella *et. al* 2010). GABA may therefore have several effects in *P. syringae* plant interactions.

In this study we investigated the growth of DC3000 and Δ*gabT* on *A. thaliana* ecotype *Ler* and *hpl1* and the response of the plants to pathogen infection. *hpl1* lacks the ability to produce green leaf volatiles (GLVs) due to a mutation in the *hydroperoxide lyase* gene. Three leaves of 4-week old plants were inoculated with either a low bacterial dose (OD_{600} of 0.0007) or a high dose (OD_{600} of 0.007) for bacterial assays and for jasmonic acid (JA), salicylic acid (SA) and GABA quantification, respectively. JA, SA and GABA were extracted from ground leaf material, homogenized in 70% methanol spiked with D_6-GABA, D_6-JA and D_6-SA (internal standards) and were quantified using LC-MS.

The growth of DC3000 and Δ*gabT* differed slightly in the different plants. The levels of GABA and hormones were different in the interaction of the different plants and bacterial genotypes and therefore we are assuming that GLVs might be involved in the interaction.

This work was supported by a scholarship from Ministry of education, culture, sports, science & Technology of Japan (MEXT), Core-to-Core project from JSPS, Faculty of Agriculture, Department of Biological Sciences, Yamaguchi University and NWO-ALW grant 818.02.017 to Robert Schuurink.

References: D.H. Park, R. Mirabella, P.A. Bronstein, G.M. Preston, M.A. Haring, C.K. Lim, A. Collmer and R.C. Schuurink, Mutations in γ-aminobutyric acid (GABA) transaminase genes in plants or *Pseudomonas syringae* reduce bacterial virulence, *Plant J* 64 (2010), pp. 318–330.

Saccharothrix algeriensis NRRL B-24137: a new endophyte with high potential to induce systemic resistance in *Vitis vinifera* L. towards *Botrytis cinerea* and to understand mechanisms of ISR in *Arabidopsis thaliana*

Presenting author: **Saima Muzammil** (saimamuzammil83@yahoo.com)

Authors: Muzammil S[1*], Graillon C[1*], Mathieu F[1], Lebrihi A[1] and Compant S[1, 2‡]

[1]Université de Toulouse, LGC UMR 5503 (CNRS/INPT/UPS), Dépt Bioprocédés et Systèmes Microbiens, ENSAT-INP de Toulouse, 1 Avenue de l'Agrobiopôle, B.P. 32607, F-31326 Castanet-Tolosan Cedex 1, France

[2]Université de Toulouse, Centre de Viticulture-Œnologie de Midi Pyrénées, Avenue de l'Agrobiopôle BP 32607, F-31326 Auzeville-Tolosane, Castanet-Tolosan Cedex 1, France.

‡Corresponding author. Dr. Stéphane Compant. E-mail: scompant@ensat.fr

*authors contributed equally

44

Since times of M.L.V. Gallipe and L. Hiltner, a lot of interesting researches have studied rhizo- and endophytic bacteria. Bacterial colonization processes on and inside plants as well as impacts of some strains on their hosts to protect them towards phytopathogens were particularly studied. However for some bacterial taxa, researches related to are still missing. In this way a rare actinobacterium from the family *Actinosynnemataceae, Saccharothrix algeriensis* NRRL B-24137 that was isolated from desert soil has been evaluated to protect plants towards *Botrytis cinerea* infection and colonization. This was studied both with *Arabidopsis thaliana* and *Vitis vinifera* L.

Data show firstly that on grapevine the bacterium can colonize beneficially plants roots both in the rhizosphere and inside endorhiza. The bacterium cannot reach the systemic plant parts but protect leaves to gray mould disease caused by *B. cinerea* by inducing mechanisms of resistance and these mechanisms are currently under investigations.

To study putative phenomenon of SAR or ISR involved, the model plant *Arabidopsis thaliana*, for which constitutive expressers of induced resistance and mutants impaired in expression of SAR and/or ISR were available, was further used. However the colonization processes of the beneficial bacterium was firstly determined and then mechanisms of resistance were studied. Data show that strain NRRL B-24137 colonize the rhizo and endosphere of *A. thaliana* in a same way as for grapevine plants. Different *A. thaliana* mutants were then used to investigate the expression of different genes involved in ISR or SAR in leaves of *Arabidopsis.* Result show that the phenomenon of resistance corresponds to an ISR. By using different plant mutants, some new components of mechanisms of ISR induced by beneficial bacteria were additionally revealed with strain NRRL B-24137, and can be added to the model proposed of ISR for a better knowledge of rhizo-/endophyte-plant association.

Salicylic acid signals through two conserved domains in the C-terminal thirds of NPR1 and some NPR1-like proteins

Presenting author: **David Neeley** (neeley@gmx.de)
Authors: Neeley D, Maier F, Pfitzner UM
Universität Hohenheim, Institut für Genetik, FG Allgemeine Virologie, D-70593 Stuttgart

45

NON-EXPRESSOR OF PATHOGENESIS-RELATED GENES1 (NPR1) is a central regulator of systemic acquired resistance (SAR) in Arabidopsis (Cao *et al*., 1997; Ryals *et al*., 1997). The NPR1 protein is activated upon SAR induction (Cao *et al*., 1998; Friedrich *et al*., 2001). Using a yeast heterologous system, we have established that NPR1 itself is likely to be a target for the SAR signal molecule salicylic acid (SA; Maier *et al*., 2011). Addition of SA to the yeast culture medium alters the biochemical capabilities of NPR1 in one-hybrid (Y1H) and Y2H assays. Whereas SA supplementation enhances transcriptional activity in tobacco NPR1, its interaction with NIM1-INTERACTING (NIMIN)2-type proteins is suppressed. The SA signal is transmitted through two highly conserved domains present in the C-terminal thirds of NPR1 proteins from multiple plant species. One domain comprises the penta-amino acid motif LENRV. This domain may serve as a hinge region. The second domain comprises the binding site for the SA-induced NIMIN1 and NIMIN2 proteins. NPR1-related proteins that contain these two conserved C-terminal domains are likewise sensitive to SA (Maier *et al*., 2011). Our findings are corroborated by an *in planta* screen for Arabidopsis mutants insensitive to the functional SA analog BTH (Canet *et al*., 2010). Of 25 *npr1* alleles with point mutations identified, 17 alleles carry amino acid substitutions in the two conserved C-terminal domains. The *nim1-4* mutant, which is impaired in SA-induced expression of the *PATHOGENESIS-RELATED (PR) PROTEIN1* gene and in mounting the SAR response (Ryals *et al*., 1997), was isolated three times. *nim1-4* contains an R to K exchange in the LENRV domain. In the yeast systems developed by us, NIM1-4 mutant proteins from Arabidopsis and tobacco are non-responsive to SA. The phenotypes of different *npr1* SA signaling mutants will be discussed as revealed by biochemical analyses in yeast.

Canet *et al.* (2010) Plant, Cell & Environ. 33, 1911-1922.
Cao *et al.* (1997) Cell 88, 57-63.
Cao *et al.* (1998) PNAS 95, 6531-6536.
Friedrich *et al.* (2001) MPMI 14, 1114-1124.
Maier *et al.* (2011) MPP 12, 73-91.
Ryals *et al.* (1997) Plant Cell 9, 425-439.

Indications for resistance induction by *Glycyrrhiza glabra* (licorice) leaf extract in cucumbers

Presenting author: **Marc Orlik** (marc.orlik@jki.bund.de)

Authors: Scherf A[1], Orlik M[1], Poidevin S[2], Mauch-Mani B[2] and Schmitt A[1]

[1]Julius Kühn-Institute, Institute for Biological Control, Darmstadt, Germany

[2]University Neuchâtel, Institute of Botany, Laboratory of molecular and cell biology, Neuchâtel, Switzerland

46

We have previously shown that the application of an ethanolic leaf extract of *Glycyrrhiza glabra* (licorice) to cucumbers grown in greenhouses under semi-commercial conditions led to a reduction of downy mildew (*Pseudoperonospora cubensis*) of up to 80% (Scherf et al 2010). *In vitro* trials revealed a direct negative effect of the extract on the release of zoospores.

Here, we report on preliminary trials set-up to test our hypothesis, that licorice extract and its active fraction (F6) exert a dual mode of action: in addition to the inhibition of zoospore release the extracts lead to the induction of systemic resistance in the treated plants.

The treatment with the full extract and fraction F6 resulted in a reduction of germ tube length of *P. cubensis* on treated cucumber leaves. Furthermore, treatment with the licorice extract and fraction F6 led to the accumulation of H_2O_2 in cucumber leaf discs as revealed by DAB-staining. The highest DAB signal was observed after 6 to 7 hours of treatment (without inoculation). Together, these results point to a possible inducing effect of the treatments.

However, the strongest indication for a direct induction of resistance in cucumbers is the accumulation of the pathogenesis-related protein PR-1. It was approximately 4 times up-regulated in licorice-treated plants compared to water-treated plants (without inoculation).

Our results suggest that in addition to the known direct effect of licorice extract on *P.cubensis,* induced resistance is a second mode of action important for the potential of the *G.glabra* extract as an alternative control agent.

In order to investigate involved signal transduction pathways, licorice extract was applied to signaling mutants of *Arabidopsis thaliana* and their resistance reaction towards *Hyaloperonospora arabidopsidis* was assessed. Neither jasmonic acid, salicylic acid nor abscisic acid seemed to be involved in the observed induction of resistance.

The results will serve as basis for in-depth investigations in a follow-up project.

Scherf, A., Schuster, C., Marx, P., Gärber, U., Konstantinidou-Doltsinis, S.†, Schmitt, A. (2010). Control of downy mildew (*Pseudoperonospora cubensis*) of greenhouse grown cucumbers with alternative biological agents. Communications in Agricultural and Applied Biological Sciences 75(4):541-554.

Defensive responses of lima bean plants against two different strains of Kanzawa spider mites

Presenting author: **Rika Ozawa** (ozawar@ecology.kyoto-u.ac.jp)

Authors: Ozawa R[1], Matsushima R[2], Ueda H[1,3], Takemoto H[1], Matsuda K[3], Maffei ME[4], Takabayashi J[1]

[1]Center for Ecological Research, Kyoto University, Japan

[2]Institute of Plant Science and Resources, Okayama University, Japan

[3]Faculty of Agriculture, Kinki University, Japan

[4]Department of Plant Biology and Centre of Excellence CEBIOVEM, University of Turin, Italy

47

In response to herbivory, plants emit a specific blend of volatiles that attracts natural enemies of herbivores. In most cases, the emission of the volatiles, so called herbivore-induced plant volatiles (HIPV), is considered as one of the induced indirect defenses of plants against herbivores. Kanzawa spider mites (*Tetranychus kanzawai*) are polyphagous herbivores that feed on various plant families including Fabaceae. We found that the two strains of the spider mite were able to induce different blends of HIPV in lima bean plants (*Phaseolus lanatus*) infested by either strain. They were divided genetically according to the color of their scars: brown-red scars by a strain “Red” and white scars by a strain “White”. To clarify how plants regulate production of specific HIPV, we investigated the responses of lima bean plants that were infested by the Red or the White. Higher expression levels of gene for acidic chitinase (acidic PR gene) that was activated by salicylic acid (SA) were detected in Red-infested leaves, while expression levels of gene for basic chitinase (basic PR gene) that was activated by jasmonic acid were similar in the leaves infested by either the Red or the White. This was in line with the observation that SA was specifically accumulated in Red-infested leaves. Furthermore, we assessed early events involved in Ca^{2+} influx and H_2O_2 generation, by a confocal laser scanning microscopy and analysis of expression of related genes (eg. *SOD, superoxide dismutase; CAT, catalase and PER, peroxidase*). Based on the results from the above analyses, we propose that two genetically different strains of the mite activate different signaling pathways leading to a mite strain-specific blend of HIPV in lima bean plants.

A specific homeostasis between callose and H_2O_2 is needed for an intact BABA-IR against *P cucumerina*

Presenting author: **Victoria Pastor** (victoria.bitacora@gmail.es)
Authors: Pastor V[1], Luna E[2], Ton J[2], García-Agustín P[3], Cerezo M[1], Flors V[1]
[1]Metabolic Integration and Cell Signaling Group. Plant Physiology Section. Department of CAMN. Universitat Jaume I. 12071. Castellón. Spain.
[2] Department of Biological Chemistry, Rothamsted Research, Harpenden AL5 2JQ U.K.
[3]Biochemistry and Biotecnology Group. Plant Physiology Section. Department of CAMN. Universitat Jaume I. 12071. Castellón. Spain.

48

Upon appropiated stimulation plants can activate the basal defense mechanisms by reacting faster and stronger to a biotic challenge reducing the chances for pathogen invasion. Among many responses, the production of reactive oxygen species (ROS) and callose deposition are the earliest events in defense. In the present work, we study the ROS and callose interplay during β-aminobutyric acid (BABA)-induced priming against the necrotrophic fungus *Plectosphaerella cucumerina*. Mutants affected in cell redox homeostasis show differences in callose depositions, as well as in basal and induced defense responses. Our findings suggest that *PAD2*, which encodes a enzyme for the first step of synthesis of glutathion (GSH) plays a relevant role in basal and induced resistance since *pad2* is hypersensitive and impaired in BABA-IR against the necrotroph. In addition, *pad2* has a reduced callose deposition and ROS production around the site of infection. On the contrary, its partner in the cycle of Halliwell-Asada, *VTC1* which participates in the synthesis of ascorbic acid (ASA), is not necessary for BABA-induced resistance (BABA-IR), since *vtc1* displays intact induced resistance. It is noteworthy, that the *vtc1* accumulates more callose than wild type upon infection. Resistance to *P. cucumerina* seems to be independent of indolic glucosinolates, since the mutant *pen2-2* behaves as wild type. On the other hand, the mutant *rbohD*, which shows low levels of H_2O_2, is not altered in basal resistance but is blocked in BABA- induced resistance. Furthermore, the mutants *cat2* and *vtc1* display normal basal and BABA-induced resistance, probably because they have enough hydrogen peroxide to provide resistance against that pathogen accumulating more callose in the apoplast which may be further stimulated by BABA to express enhanced resistance. In conclusion, a specific redox regulation is necessary for BABA-IR against *P. cucumerina.*

Dominant mutations in Arabidopsis shed light on how CAMTA3 regulates defence signalling

Presenting author: **Carsten Pedersen** (cpr@life.ku.dk)
Authors: Pedersen C[1], Andersson MX, Pagter M, Nielsen ME, Lenk A, Zhang Z, Thordal-Christensen H
[1]Department of Agriculture and Ecology, University of Copenhagen

49

In a mutant suppressor screen, we identified two (semi)dominant mutants rescuing the lesion-mimic phenotype of the syntaxin syp121 syp122 double mutant and map-based cloning showed that they were mutated in separate sites of the IQ-domains of CAMTA3, a CaM-binding transcription activator. The CAMTA3 T-DNA loss-of-function mutant itself has a weak lesion-mimic phenotype and a constitutive activation of defence signalling. Hence it increases resistance toward bacterial and fungal pathogens. Thus in our dominant CAMTA3 mutants we see the opposite effect of knocking out the gene. Consistently, the dominant mutations in wild-type syntaxin gene background led to increased disease susceptibility. Taken together, the phenotypes of the two types of mutations in CAMTA3 show that the function of the transcription activator is to suppress pathogen defence signalling and that this suppression becomes constitutive after mutations in the IQ-domain, known to be involved in calmodulin-binding.

The weak lesion-mimic phenotype of the loss-of-function mutant can be rescued by combining it with mutations in genes acting as positive regulators of defence signalling. Likewise, we combined the dominant CaM-domain mutant with other suppressors of syntaxin-related death in the syp121 syp122 background and found that this always led to further rescue of the lesion-mimic phenotype. This indicates that CAMTA3 acts at a basal level in defence signalling. Since recently published work has shown involvement of CAMTA3 in cold acclimation, we have also studied the influence of the dominant mutation and loss-of-function mutation on this phenotype.

Functional and molecular analysis of grapevine induced resistance against *Plasmopara viticola* in relation to the physiology of the microbial inducer

Presenting author: **Michele Perazzolli** (michele.perazzolli@iasma.it)

Authors: Vincenot L, Pertot I, Perazzolli M

[1]IASMA Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, S. Michele all'Adige 38010, Trento, Italy

50

Trichoderma harzianum T39 is a biocontrol agent active against grapevine downy mildew (*Plasmopara viticola*). Its biocontrol activity is mainly based on the induction of plant resistance. However, as most of biocontrol agents, its efficacy is often inconsistent, especially under field conditions. The differences in efficacy can be related to the physiological status of the plant, the environmental conditions (as temperature, relative humidity, leaf wetness) or the physiology of the microorganism. For some biocontrol agents, the nutrition affects conidia production and quality, which could be particularly important for the biocontrol activity. Our aim was to test if the physiological stage of conidia modulates the efficacy of T39, in the tripartite interaction *T. harzianum – P. viticola – Vitis vinifera* under controlled greenhouse conditions.

We evaluated the level of grapevine resistance against *P. viticola* induced by T39 at different conidial age produced on different substrates. We examined i) the direct antagonistic activity of T39 against *P. viticola* inoculum by competition or inhibition of the pathogen germination and ii) the ability of T39 to induce grapevine local and systemic resistance against the pathogen. Resistance induction was characterized in terms of modulation of disease-related genes in plants treated or not with the biocontrol agent produced under different conditions.

Our study revealed a comparable efficacy of T39 conidia produced on different media, and a trend of higher biocontrol activity by younger conidia. Microscopic observations showed that efficacy of young conidia is not related to an increased germination rate. No evidence of direct antagonism against the pathogen suggests major implication of resistance induction. Grapevine defence response was characterized in terms of modulation of disease-related genes in plants treated or not with the biocontrol agent produced under different conditions.

Our results could be useful to optimise the production of highly active *T. harzianum* T39 conidia for plant resistance induction.

Effect of environmental factors on the interaction Plant-Pathogen-*Bacillus amyloliquefaciens* S499

Presenting author: **Ilaria Pertot** (ilaria.pertot@iasma.it)

Authors: Pertot I[1], Hosni T[1,2], Pedrotti L[1], Puopolo G[1], Jourdan E[2], Ongena M[2]

[1]IASMA Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, S. Michele all'Adige 38010, Trento, Italy

[2]Gembloux Agro-Bio Tech, University of Liège, Passage des Déportés, 2, Gembloux, Belgium

51

As living organisms, growth and antagonistic activity of biological control agents depend on environmental conditions. Unfortunately, unsatisfactory or inconsistent disease biocontrol has frequently been reported under field conditions. Although relevant, the effect of environment factors on some biocontrol mechanisms is far from being full understood. Temperatures and water stress are generally considered the most detrimental factors for survival and activity of microbial biocontrol agents.

Bacillus amyloliquefaciens S499 (S499) can readily colonize the rhizosphere of many plants and induce systemic resistance (ISR) against several pathogens. While the role of surfactin production by S499 in ISR is clearly demonstrated, the influence of environmental factors on this biocontrol mechanism is still unclear. The aims of our research were to understand the effect of temperature and water stress on systemic resistance induced by S499. We evaluated the effect of temperature on S499 growth and lipopeptide production and exposure of plants to temperature and water stress prior and after S499 inoculation on the level of ISR.

Under laboratory conditions, a marked influence of temperature on growth and production of lipopeptides by strain S499 was observed. Final cell densities were globally similar in cultures performed at 15, 20, 30, 40 and 48°C, but growth at low temperatures (15 and 20°C) was characterized by a much longer lag-phase, probably necessary for cell adaptation. Higher concentrations of surfactin were also measured after growth at 15 and 20°C compared to 30°C and the lipopeptide production is clearly impaired at higher temperatures. The effect of temperature (15, 25 and 35°C) and water stress on ISR, was evaluated on zucchini, bean and tomato inoculated with the pathogens (*Podosphaera xanthii*, *Botrytis cinerea*, and *Phytophthora infestans* on zucchini, bean and tomato, respectively). A short exposure of plants to temperature and water stress reduced the level of ISR on plants and influenced at different extent the root colonization on the three crops. Even if exposure to temperature and water stress generally reduced the level of ISR on the three crops, the highest reduction was found on zucchini.

This is the first study of an interaction between plants, bacteria and ISR under temperature and water stress conditions.

Elicitors: a promising strategy in plant protection

Presenting author: **Adeline Picot** (picot@vegenov.com)

Authors: Guerrand J[1], Monot C[1], Aljabal M [1], Menil A[1], Picot A[1] and Hallier S[1]

Vegenov-BBV, Penn ar Prat, F-29 250 St Pol de Léon

hallier@vegenov.com

52

To face our challenging goals in regards to integrated plant protection, Vegenov proposes since more than 15 years its scientific and technical expertises in the development of alternative solutions through interdisciplinary approaches, such as disease resistance and plant treatments with elicitors. Regarding elicitors, we work both on efficiency and mode of action, including their ability to stimulate plant defence, their possible biocide effect, their persistence of action, and their systemy in the plant. This poster presents a summary of some of our recent results under controlled conditions. Those data show the interest of using elicitors as complementary strategy in integrated pest management. Results on persistence of action reveal the ability of some elicitors to protect the plant over a long period of time. Current projects are on their ways to better understand the impact of plant physiology and environmental factors such as plant genetic, plant stage, nutrition, and abiotic stresses on elicitor efficiency.

Induced systemic resistance in maize

Presenting author: **Chantal Planchamp** (chantal.planchamp@unine.ch)

Authors: Planchamp C[1], Balmer D[1], Robert CAM[2], Zwahlen C[2] and Mauch-Mani B[1]

[1]University of Neuchâtel, Department of Biology, Laboratory of Molecular and Cellular Biology, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland
[2]University of Neuchâtel, Department of Biology, Laboratory for Fundamental and Applied Research in Chemical Ecology (FARCE), Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland

53

Induced systemic resistance (ISR) is elicited by non-pathogenic root-colonizing bacteria or fungi and is efficient against pathogens and insects. Despite the economic importance of maize, studies about its defenses are less common than studies about induced resistance involving dicotyledonous plants. The goal of the present study consists in a better understanding of the mechanisms that lead to defense reactions induced by beneficial bacteria in maize. For this purpose, various parameters affected by *Pseudomonas putida* KT2440 have been tested in maize plants. Root inoculation of maize seedlings with these bacteria induced defense reactions in leaves against the hemibiotrophic pathogen *Colletotrichum graminicola*, the causal agent of corn anthracnose, showing the potential of *P. putida* KT2440 to induce resistance in maize plants. Moreover, experiments have been conducted to test the effect of the presence of *P. putida* KT2440 on the leaf herbivory. Plants were infested with larvae of *Spodoptera littoralis*, a generalist herbivore, and *Spodoptera frugiperda*, a specialist herbivore. There were no differences in larval weight gain in the case of the specialist *S. frugiperda* but larvae of the generalist *S. littoralis* grew better on untreated plants. These results indicate that in addition to inducing resistance against *C. graminicola* the bacteria induce anti-herbivore defense in the plants. Interestingly this defense is effective against the generalist but seems to be suppressed or coped with by the specialist. Further studies to better understand these induced resistance mechanisms are currently in process.

Metabolite profiling and feeding bioassays suggest a major role for a dicaffeoylquinic acid in induced resistance of peach to *Myzus persicae* aphid

Presenting author: **Jean-Luc Poëssel** (Jean-Luc.Poessel@avignon.inra.fr)

Authors: Poëssel JL[1], Sauge MH[2], Staudt M[3], Dufour C[4], Deborde C[5,6], Rahbé Y[7], Jackson B[3], Renaud C[8], Maucourt M[6,9], Corre MN[1], El-Aouni H[3], Lacroze JP[2], Moing A[5,6]

[1]INRA, UR 1052, Génétique et Amélioration des Fruits et Légumes, Avignon, France
[2]INRA, UR 1115, Plantes et Systèmes de culture Horticoles, Avignon, France
[3]CEFE-CNRS, Montpellier, France
[4]INRA, UMR 408, Sécurité et Qualité des Produits d'Origine Végétale, Avignon, France
[5]INRA, UMR 1332 Biologie du Fruit et Pathologie, Bordeaux, France
[6]Plateforme Métabolome du CGFB, IBVM, Bordeaux, France
[7]INRA, UMR 203, Biologie Fonctionnelle, Insectes & Interactions, Lyon, France
[8]INRA, UR 419, Unité de Recherches sur les Espèces Fruitières, Bordeaux, France
[9]Université de Bordeaux, UMR 1332 Biologie du Fruit et Pathologie, France

54

The green peach aphid (GPA, *Myzus persicae* Sulzer) is a generalist insect pest infesting agricultural and horticultural crops worldwide. GPA causes significant direct damages to peach, its primary host, and may transmit viruses that cause serious diseases such as Sharka conferred by the *Plum Pox Potyvirus*. Resistant genotypes have been identified among the peach germplasm and are used in breeding programs to create peach cultivars showing a durable resistance to GPA. Among these genetic resources, Rubira, a red-leaf cultivar used as rootstock and carrying the dominant resistance gene *Rm2*, shows a strong and induced antixenosis-type resistance associated with hypersensitive-like necrotic lesions on developing leaves.

To study the mechanisms involved in induced resistance of Rubira to GPA, we used a metabolomics approach and investigated metabolite changes occurring in shoot apices of Rubira and a susceptible cultivar after a 48-hours infestation with GPA. Untargeted ^{1}H NMR and targeted LC (sugars, organic acids, amino acids), LC-MS (secondary metabolites including phenolic and cyanogenic compounds) and GC-MS (Volatile Organic Compounds, VOCs) were applied. While no significant modifications occurred in the susceptible cultivar, dramatic changes in primary and secondary metabolites were observed in Rubira following infestation. Carbohydrates and most organic acids showed a marked decrease. Several amino acids, including lysine and branched-chain and aromatic amino acids, showed a large accumulation whereas levels of glutamine, proline and threonine were greatly reduced. Infestation of Rubira by GPA also triggered the release of VOCs, mainly methyl-salicylate and (E,E)-α-farnesene, and the accumulation of secondary metabolites. Chlorogenic acid (5-caffeoylquinic acid, 5-CQ) and 3,5-dicaffeoylquinic acid (3,5-diCQ) were the main phenolics of shoot apices. Strikingly, these closely related compounds showed differential responses to infestation: while 5-CQ level did not change, 3,5-diCQ showed a significant accumulation and therefore could be involved in resistance. The effect of 5-CQ and 3,5-diCQ on GPA was further studied in bioassays with artificial diets. Whereas 5-CQ did not show any effect on larval development, 3,5-diCQ was highly toxic and larvae did not survive at the concentration as low as 1mM (516 mg.L^{-1}). These results suggest that 3,5-diCQ could play a major role in antixenosis resistance of Rubira to green peach aphid.

New insights into innate immunity in Arabidopsis: discovery of a LOS signalling network by multidisciplinary approaches

Presenting author: **Silvia Proietti** (s.proietti@unitus.it)

Authors: Proietti S[1], Bertini L[1], Amoresano A[2], Giangrande C[2], Caporale C[1] and Caruso C[1]

[1]Department of Ecological and Biological Sciences, Università della Tuscia, 01100 Viterbo, Italy
[2]Department of Organic Chemistry and Biochemistry, Università di Napoli Federico II, 80126 Napoli, Italy

55

Plants possess an innate immune system enabling them to defend themselves against pathogen attack. This constitutive process becomes a major factor in their survival when the enemy is not deterred by the chemical and physical barriers of the plant. The plant immune system perceives the presence of pathogens by recognition of molecules known as pathogen-associated molecular pattern (PAMP) or by sensing effector proteins that are secreted by the host during plant–pathogen interactions. Early interactions between PAMP and cell surface receptors are among the first line of recognition events leading to appropriate defences by activating a multicomponent and multilayered response. The induction of defence is triggered by complex pathways that can involve Ca^{2+} influx, H^+/K^+ exchange, generation of reactive oxygen and nitrogen species (ROS and RNS, respectively) and the synthesis of jasmonic acid (JA), salicylic acid (SA) and ethylene (Et), which act in a multifaceted cross-talk manner as signal molecules. The inducible plant defence responses are characterized by defence gene activation, activation of programmed hypersensitive cell death responses, production of antimicrobial phytoalexins, strengthening of the cell wall and synthesis of pathogenesis-related (PR) proteins.

In bacteria, the most important PAMPs are represented by conserved cell-surface structures like flagellin, lipopeptides (LP), peptidoglycanes (PG) and lipopolysaccharides (LPS). LPS from plant-associated and plant pathogenic bacteria possess the same tripartite structure comprising lipid A, core oligosaccharide (OS) and an O-polysaccharide or O-antigen. The lipid A and the core OS are linked in the majority of cases by the sugar 3-deoxy-D*manno*- oct-2-ulosonate (Kdo). LPS molecules that lack an O-antigen are called lipooligosaccharides (LOS). Recognition of LOS in mammals is rather complex; in plants the mechanism of this recognition and consequent transduction steps remain obscure.

Here, we aim to shed some light on the signalling network in *Arabidopsis thaliana* following the LOS-induced defence response, by proteomic and transcriptomic approaches. By using a proteomic approach based on pull-down strategies and tandem mass spectrometry analysis, we have identified protein ligands of LOS from *Xanthomonas campestris* pv. *campestris* (*Xcc*), the causative agent of black rot, a disease of cruciferous crops that is of worldwide importance. *Xcc* can also infect non-crop crucifers, such as Arabidopsis. The results show a complex pattern made by proteins involved in cellular metabolism, defence and signal transduction. Focusing our analysis on genes encoding transcription factors, PR proteins, PAMP recognition and signal transduction molecules, we aim to monitor their expression in Arabidopsis plants elicited by LOS infiltration. Our results, still in progress, will contribute to define the recognition strategy of pathogen and its signal transduction pathway.

PR4 protein from Arabidopsis: its role on plant defence and its potential biotechnological use

Presenting author: **Silvia Proietti** (s.proietti@unitus.it)

Authors: Proietti S[1], Bertini L[1], Mondello F[2], Girolamo A[2], Cassone A[2], Caporale C[1] and Caruso C[1]

[1]Department of Ecological and Biological Sciences, Università della Tuscia, 01100 Viterbo, Italy

[2]Department of Infectious, Parasitic and Immune-mediated Diseases, Istituto Superiore di Sanità, Viale Regina Elena 299, 00161 Rome, Italy

56

Plants possess both preformed and inducible mechanisms to resist pathogen invasion. Pre-existing morphological barriers, secondary metabolites, and antimicrobial proteins must be eluded by pathogens in order to invade a plant. Once contact has been established, elicitors produced and released by the pathogen induce further defences, comprising the reinforcement of cell walls, the production of phytoalexins, and the synthesis of defence-related proteins. Most of these resistance proteins correspond to pathogenesis-related proteins (PRs), which were identified several years ago as being associated with active defence and resistance to various pathogens. It is widely recognized that PR proteins play a role in restricting pathogen development and plant invasion. Biological activity and function are already known for most PR protein classes, but not for all of them. In the last years, we focussed our attention on PR proteins of class 4 (PR4), among the less studied. We have isolated and sequenced four PR4 proteins from wheat kernels, named wheatwin1 to wheatwin4, that strongly inhibit both host and non-specific phytopathogenic fungi. Moreover, we have demonstrated that they are endowed with ribonuclease activity, highlighting a strong correlation with the antifungal activity by producing mutant proteins affecting specific amino acids of the active site.

We already reported the isolation of the PR4 ortholog gene from *Arabidopsis thaliana,* named AtHEL. Its transcriptomic profile following pathogen infection, treatment with phytohormones and wounding suggest a role in plant defence responses to several stresses.

In this work, we studied the cellular localization of the mature HEL protein by transient expression assay using protoplast transformation. Moreover, AtHEL protein produced at high level in a recombinant form was found to possess ribonuclease and antifungal activities stronger than those shown by the wheatwins. The growing interest around the research of new natural bioactive molecules prompted us to test the activity of this recombinant protein against human pathogens. Tests carried out on *Candida albicans*, the main etiologic agent of opportunistic mucosal and systemic infections in humans, showed that recombinant AtHEL has a candidacidal effect on budding cells at a reasonably low protein concentration (minimal fungicidal concentration: averages 4 ug/ml). To the best of our knowledge, this is the first time that PR4 proteins have been shown to be active against a human pathogen. These results are of relevance to the exploitation of this molecule in biotechnological process, in view of a larger use of bio-friendly plant based products.

Update on crosstalk between salicylic acid and jasmonate in defense signaling: a proteomic approach

Presenting author: **Silvia Proietti** (s.proietti@unitus.it)

Authors: Proietti S, Bertini L, Timperio AM, Caporale C, Caruso C

Department of Ecological and Biological Sciences, Università della Tuscia, 01100 Viterbo, Italy

57

In nature, plants often deal with simultaneous invasion by multiple aggressors, which can influence the primary induced defense response of the host plant. Activation of plant defense mechanisms is associated with ecological fitness costs, so plants need regulatory mechanisms to effectively and efficiently adapt to changes in their complex environment. Crosstalk between hormonal signaling pathways may have evolved to allow plants to fine-tune the induction of their defense in response to different plant pathogens. The importance of SA, JAs, and ET as dominant primary signals in local and systemic induced defense signaling has been well documented. It's known that resistance conferred by biotrophic pathogens often requires SA signalling, whereas necrotrophic pathogens or wounding mainly activate the JA/ET-dependent pathway. Thus, at least two different signal transduction pathways can be distinguished, which are turned on in response to pathogen attack. In recent years, research on phytohormones biosynthetic pathways and the way they are perceived by other bio-molecules significantly advanced our understanding on the signaling pathways activated by these small molecules. However, the way these signal molecules function in a complex network of interacting signaling pathways is less well known and deserves in-depth.

Currently, interconnected signalling networks between the above phytohormones have been investigated using transcriptomic approach. However, transcriptional changes do not reflect the complete cellular regulatory processes, since post-transcriptional processes are not taken into account. Thus, complementary approaches such as proteome-based expression profiling are needed to obtain a full picture of the regulatory elements in plant defense response. In order to shed some light on the crosstalk between SA and JA in Arabidopsis, we have used 2D-PAGE coupled with LC-MS/MS to identify the significantly up- or down-regulated Arabidopsis leaf proteins upon simultaneous treatment with both phytohormones, respect to single treatments. Most of the 30 selected protein spots successfully identified by Mascot analysis have functions related to stress response while the other are involved in physiological processes as energy production. These results increase our understanding on metabolic changes following crosstalk between defense hormones and help to clarify its biological significance.

Induction of acquired resistance of tomato plants to *Fusarium oxysporum* by hydrogen peroxide

Presenting author: **Natallia Pshybytko** (pshybytko@rambler.ru)

Authors: Pshybytko NL[1], Zhavoronkova NB[1], Zenevich LA[1], Kabashnikova LF[1] and Lysenko EA[2]

[1]Institute of Biophysics and Cell Engineering, National Academy of Sciences of Belarus

[2]Institute of Plant Physiology, Russian Academy of Sciences

58

The main physiological processes in tomato plants under pathogenesis of *Fusarium oxysporum* were studied. Pathogens were inoculated in 4-mounts-old tomato plants trough root system. The pathogenesis carried out about 30-40 days and drying up of tomato plants was watched after them yellowing. The pathogenesis was caused by toxins of *Fusarium oxysporum*, which suppressed the physiological processes in tomato plants and inhibited biosynthesis of main cell components. Moreover the fungi proliferated actively in root tissues and mycelium grow, enlarged and corked the phloem. The decrease of xylem flow and dehydration of plant tissue were displayed. The activation of destructive processes (lipid peroxidation and generation of reactive oxygen) was obtained. Some peaks of ROS generation were shown after *Fusarium oxysporum* inoculation. First peak was identificated in half an hour after infection, and physiological processes in tomato plant were not changed. Repeated increase in ROS generation was after 5-7 days. In this case destructive processes was accelerated and some physiological functions were limitated. The PR proteins accumulation was indicated after 24 h. Thus, we surmise that first ROS burst is signal for activation protective mechanisms while second ROS accumulation is destructive process.

For the purpose of induction of acquired resistance of plants to pathogens the pretreatment of plants growing in hydroponics by exogenous hydrogen peroxide was tested. Exogenous hydrogen peroxide induced 4 times increase in endogenous ROS content after 3 h after treatment. Then ROS level decreased but it was at high level over a long period of time. In this time the PR proteins synthesis accelerated and pretreatment plants had high level of chitinase. Under following infection of plants pathogen could not penetrate through defence barriers or it development was suppressed. The mechanisms of plant – fungi interaction, signal transduction and hydrogen peroxide role in plant protection are discussed.

Characterization of *Trichoderma harzianum* T39 induced resistance against *Plasmopara viticola* in heat and drought stressed grapevine plants

Presenting author: **Benedetta Roatti** (benedetta.roatti@iasma.it)

Authors: Roatti B[1,2], Perazzolli M[1], Giovannini O[1], Pertot I[1]

[1]IASMA Research and Innovation Centre, Fondazione Edmund Mach, Via E. Mach 1, S. Michele all'Adige 38010, Trento, Italy

[2]Institute of Integrative Biology, Swiss Federal Institute of Technology (ETH), Zurich, 8092, Switzerland

59

Plants can acquire an enhanced defensive capacity after treatment with a resistance-inducing agent that results in a faster and/or stronger defence reaction against pathogens. These agents induce a broad spectrum and long lasting resistance, but rarely provide complete control of infections (for the most part between 20 to 85% of disease control). Under field conditions, the expression of induced resistance is often inconsistent and likely to be influenced by the environment, plant genotype and its physiology. However little information is available on the role of these factors on the level of induced resistance. Since abiotic stresses may strongly influence the host plant, the aim of this project is to investigate the effect of a short heat and/or drought exposure of grapevine plants on the systemic resistance activated by *Trichoderma harzianum* T39 (T39) or benzothiaziadole (BTH) against downy mildew (*Plasmopara viticola*).

In susceptible grapevine cultivars grown under controlled greenhouse conditions, foliar treatments with T39 or BTH reduce disease severity. Gene expression analysis indicates the activation of jasmonic acid and ethylene signal in the defence induced by T39, while salicylic acid pathway is activated by BTH. The pre-exposure of plants to drought and/or heat stress (abiotic stress) did not affect the efficacy of BTH-activated resistance. On the contrary, the T39-induced resistance was influenced by the plant pre-exposure to the above mentioned abiotic stress conditions, showing different levels of downy mildew reduction.

The results indicate that T39-induced resistance might be a useful tool in the control of downy mildew, but, even if its efficacy level is not affect by the genotype, it can be modulated by the physiological state of the plant. Further characterization of molecular events of T39-induced resistance under abiotic stress exposure could be particularly important in order to predict the possible effect and stability of T39-induced resistance under field condition especially in a view of climate change.

Benzoxazinones are constitutive defense in roots but inducible in leaves

Presenting authors: **Christelle A. M. Robert & Gaëtan Glauser** (christelle.robert@unine.ch)

Authors: Robert CAM[1+], Glauser G[1,2+], Gaillard MD[1], Marti G[3], Erb M[1,4], Turlings TCJ[1]

[1]University of Neuchâtel, Laboratory for Fundamental and Applied Research in Chemical Ecology (FARCE), Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland.
[2]Chemical Analytical Service of the Swiss Plant Science Web, University of Neuchâtel, Rue Emile-Argand 11, 2000 Neuchâtel, Switzerland.
[3]Phytochemistry & Bioactive Natural Products and Pharmacognosy, University of Geneva, Quai Ernest-Ansermet 30, 1211 Geneva, Switzerland
[4]Max Planck Institute for Chemical Ecology, Beutenberg Campus, Hans-Knöll-Str. 8, 07745 Jena, Germany.
[+]These authors contributed equally to the work

60

Optimal defense theory (ODT) predicts allocation of constitutive defensive chemistry within a plant as a function of the tissue value and the risk of attack. Overall, leaves and roots, both serving vital functions for a plant, can be considered as equally valuable and under comparable risks of attack by herbivores. In parallel, ODT also defines inducible defenses as dependent on the risk of future attacks. Our study aimed at screening both constitutive and inducible defensive compounds in maize organs upon above- and/or belowground herbivory. Using an untargeted metabolomic approach, we found that 1,4-benzoxazin-3-ones, including the toxic aglucone 2,4-dihydroxy-7-methoxy-1,4-benzoxazin-3-one (DIMBOA), are constitutive components of root defenses, but are also induced in leaves under herbivory. Results are presented and discussed in the context of optimal plant defense. Elucidating the physiological plant responses to crop pests will not only contribute to our fundamental understanding of insect-plant interactions, but may also help generate new avenues for pest management strategies.

Evaluation of the resistance induction in zucchini caused by foliar application of a *Nostoc* sp. extract

Presenting author: **Roberta Roberti** (roberta.roberti@unibo.it)

Authors: Roberti R[1], Righini H[1], Burzi PL[2], Perez Reyes C[3], Galletti S[2], Garcia-B. Reina G[3]

[1]Dipartimento di Protezione e Valorizzazione Agroalimentare, Alma Mater Studiorum Università di Bologna, Italia
[2]Consiglio per la Ricerca e Sperimentazione in Agricoltura, Centro di Ricerca per le Colture Industriali, Bologna, Italia
[3]Centro De Biotecnología Marina, Universidad De Las Palmas De Gran Canaria, Islas Canarias, España

61

The concern for high quality food without pesticide residues and for a sustainable agriculture that prevents environment pollution, has stimulated researches dealing with alternative control means. Cyanobacteria are components of fertilizer compounds which may interfere with plant physiology, making plants less susceptible to biotic and abiotic stresses.

The aim of this research was to study the possibility to induce systemic resistance in zucchini plants by foliar application of cyanobacterium *Nostoc* sp. extract.

Two trials were carried out under controlled conditions: the first one consisted in a biological assay of *Nostoc* extract against *Podosphaera leucotricha* and the second one in biochemical assays in order to test the involvement of induced resistance. Chitosan was used as positive control, since it is a well-known resistance inducer. *Nostoc* extract (2 g l^{-1}) was applied by spraying one of the two cotiledonar leaves in both experiments, while the pathogen was inoculated on the non-treated leaves (5 x 10^4 conidia per ml of water) in the biological assay only. Disease symptoms were recorded as percentage of infected area on non-treated leaves 7-9 dpi. For the biochemical assays, total proteins were extracted following a non-denaturing method and the expression of some PR-proteins (PR-3, PR-5 and PR-9) was tested by spectrophotometric and by isoelectrofocusing methods.

The biological assay revealed that the *Nostoc* extract reduced the disease by 20%, similarly to chitosan, with respect to the inoculated control. Both spectrophotometric and isolectrofocusing methods showed differences of the enzymatic activities tested between treated and non-treated plants, suggesting the ability of the *Nostoc* extract tested to induce systemic resistance in zucchini.

Functional genomic approaches to enhance wheat defence against biotic stress

Presenting author: **Joanne Robson** (Joanne.Robson@newcastle.ac.uk)

Authors: Robson J[1], Edwards MG[1], Ferry N[2], Kilby P[3], Crosswaithe A[3], Walling LL[4], Gatehouse AMR[1]

[1]School of Biology, Newcastle University, Newcastle upon Tyne, NE1 7RU
[2]School of Environment & Life Sciences, University of Salford, Salford M5 4WT
[3]Syngenta, Jealott's Hill International Research Centre, Bracknell, Berkshire, RG42 6EY
[4]Department of Botany and Plant Sciences, Center for Plant Cell Biology, University of California, Riverside, CA 92521

62

Aphids (Order Hemiptera) cause direct damage by feeding on crops as well as vectoring plant viruses. In Britain it has been estimated that aphids cause a mean percentage loss of 10-13% in wheat. The grain aphid [*Sitobion avenae* (F.)] is an important pest of commercially grown wheat (*Triticum aestivum*) responsible for the transmission of barley yellow dwarf virus (BYDV). There is a need to develop strategies to protect crops in a sustainable manner, particularly in light of recent EU directives banning the use of many effective synthetic pesticides. Some alternative sustainable strategies for crop protection seek to exploit the endogenous resistance mechanisms exhibited by plants to most insect herbivores. Recent studies have shown that increased levels of antioxidant enzymes involved in detoxification of reactive oxygen species (ROS) following aphid infestation may be linked to mechanisms of tolerance. In order to better understand the role of ROS in wheat's tolerance or susceptibility to *S. avenae,* the changes in the transcriptome are being investigated using the the Affymetrix GeneChip® Wheat Genome Array following aphid infestation (5 days) and 24 hour ozone treatment (positive control). Results to date show that in the susceptible cultivar (Maris Huntsman), most of the ROS enzyme transcripts are up-regulated following aphid infestation but down-regulated in response to ozone. Interestingly, following aphid infestation, no ROS enzyme transcripts were significantly differentially expressed in the tolerant cultivar (Claire). However, following ozone treatment SOD transcripts were significantly up-regulated, while catalase RNAs were down-regulated, in the aphid-tolerant Claire cultivar. Complementary enzymatic studies are being carried out and investigations at the proteome level are being performed using 2D electrophoresis and MALDI-Tof MS.

Mixtures of plant growth promoting fungi (PGPF) and arbuscular mycorrhizal fungus (AMF) *Glomus mosseae* induce resistance in cucumber plants

Presenting author: **Mary Grace B. Saldajeno** (grace.saldajeno@gmail.com)

Authors: Saldajeno MGB[1], Elsharkawy MM[1], Chandanie WA[1], Ito M[2], Shimizu M[2]

[1]The United Graduate School of Agricultural Sciences, Gifu University, 1-1 Yanagido 501-1193, Gifu City, Japan

[2]Laboratory of Plant Pathology, Faculty of Applied Biological Sciences, Gifu University, 1-1 Yanagido 501-1193, Gifu City, Japan

63

Plant growth-promoting fungi (PGPF) are saprotrophic fungi isolated from zoysia grass rhizosphere that are capable of enhancing plant growth and suppressing plant diseases. The composite application of the PGPF with the arbuscular mycorrhizal fungus (AMF) *G. mosseae* (Gm) to the soil during planting enhanced growth of cucumber, bentgrass and tomato plants and suppressed cucumber mosaic, anthracnose, damping-off, brown patch and crown and root rot diseases caused by *Cucumber Mosaic Virus* (CMV), *Colletotrichum orbiculare*, *Rhizoctonia solani* and *Fusarium oxysporum* f. sp. *lycopersici*, respectively. The enhancement in plant growth was estimated to be at the average of 1-4 folds increase over the control plants. Depending on the PGPF, Gm showed a varied tendency to depress, enhance, or no effect to the root and/or rhizosphere population of PGPF. Similarly, the PGPF reduced, enhanced or had no effect on percent root colonization of Gm. We are currently elucidating the mechanisms by which PGPF and Gm suppress symptom development of leaf pathogens like CMV and anthracnose in cucumber plants. Leaf and/or root samples were taken from cucumber grown in soil medium amended with PGPF and/or Gm at sowing at different periods after challenged inoculation with CMV or *C. orbiculare.* Preliminary results of molecular studies indicated that defense-related genes like PR1.1, Pal, β1-3 glucanase, and basic chitinase exhibited elevated expression. We hypothesize the involvement of multiple defense mechanisms leading to ISR in cucumber against CMV or *C. orbiculare*.

β-aminobutyric acid protects *Brassica napus* plants from infection by *Leptosphaeria maculans*. Resistance induction or a direct antifungal effect?

Presenting author: **Vladimír Šašek** (sasek@ueb.cas.cz)

Authors: Šašek V[1], Špácová M[1,2], Valentová O[2], Burketová L[1]

[1]Laboratory of Plant Pathophysiology, Institute of Experimental Botany AS CR, Prague, Czech Republic

[2]Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, Czech Republic

64

Resistance in plants can be induced by treatment with various chemicals. One such compound is β-aminobutyric acid (BABA). Its positive effect on disease resistance has been reported in several pathosystems. Here we demonstrate that treatment with BABA protects *Brassica napus* plants from infection by a fungal pathogen *Leptosphaeria maculans*. In order to identify the BABA mode of action we conducted series of experiments comparing the effects of BABA with a known resistance inducer benzothiadiazole. Surprisingly, BABA displayed in vitro antifungal activity against *L. maculans*. Moreover, in contrast to benzothiadiazole the effect of BABA was almost independent from the timing of treatment, indicating possible antifungal activity in planta. On the other hand, quantification of multiple hormones and expression analysis has shown that treatment with BABA induces synthesis of salicylic acid (SA) and expression of SA marker gene *PR-1*. Since BABA is known to prime plant's immune system rather than directly induce defence responses, we analysed the SA level and expression of *PR-1* also in plants pre-treated with BABA and subsequently inoculated with *L. maculans*. Nevertheless, we haven't observed any evidence for BABA-induced priming of SA responses. Although we have not clearly demonstrate whether the effect of BABA on *L. maculans* infection is based on its antifungal activity or induction of SA dependent responses, our results indicate a different mechanism from those previously identified in *Arabidopsis*.

Hexanoic acid-induced resistance against *Pseudomonas syringae* causes changes in jasmonic acid pathway

Presenting author: **Loredana Maria Scalschi** (scalschi@uji.es)

Authors: Scalschi L[1], Vicedo B[1], Sanmartin M[2], Sánchez JJ[2], González Bosch C[3], García Agustín P[1]

[1]Grupo de Bioquímica y Biotecnología Vegetal. Área Fisiología Vegetal. Departamento de Ciencias Agrarias y del Medio Natural. Escuela Superior de Tecnología y Ciencias Experimentales. ESTCE. Universitat Jaume I, Spain.
[2]Departamento de Genética Molecular de Plantas, Centro Nacional de Biotecnología (CSIC), Madrid, Spain
[3]Departamento de Bioquímica y Biología Molecular, Universitat de València, IATA (CSIC), Valencia, Spain.

65

Plants are able to develop an enhanced defence mechanism, the so-called induced resistance (IR), in addition to basal defence upon appropriate stimulation.

We have demonstrated that tomato plants treated with hexanoic acid display enhanced resistance against the *Pseudomonas syringae* (Pst) DC3000 strain. After 72 hpi, hexanoic acid treatment reduced disease symptoms and colony-forming units. To establish the mechanism involved in hexanoic acid induced-resistance (Hx-IR), different hormonal pathways were analysed. The Salicylic acid (*PR1*) and Jasmonic acid (*LoxD*) marker genes expressions significantly increased in the plants treated with hexanoic acid and infected with Pst at 48 hpi relative to the untreated plants. In addition, a hormonal analysis of hexanoic acid-treated plants revealed that OPDA accumulated faster and stronger upon infection respect untreated plants. Nonetheless, JA and JA-Ile did not increase in these plants. In order to explain the increase in both OPDA and the *LoxD* gene expression, we specifically analysed the genes involved in the JA synthesis pathway. On the other hand, our previous research has demonstrated that OPDA accumulates in tomato and Arabidopsis plants in response to *Botrytis cinerea* inoculation, and that hexanoic acid treatment raises OPDA levels 72h after fungal infection (Vicedo et al, 2009; Kravchuk et al, 2011). In order to determine the specific role that OPDA plays in Hx-IR against *Pseudomonas syringe* and *Botrytis cinerea,* we silenced the tomato gene encoding 12-Oxophytodienoate reductase 3 (OPR3).

Vicedo et al 2009. MPMI vol 22,11pág 1455-1465
Kravchuk et al, 2011. J. Plan Physiology. 168, pág. 359-366

Trehalose signaling in plant defence against sap-sucking insect

Presenting author: **Jyoti Shah** (shah@unt.edu)

Authors: Singh V[1], Louis J[1], Mondal HA[1], Nalam V[1], Ayre B[1], Reese J[2], and Shah J[1]

[1]Department of Biological Sciences, University of North Texas, Denton, TX, USA

[2]Department of Entomology, Kansas State University, Manhattan, KS, USA

66

Aphids are phloem-feeding insects that are important pests of a wide variety of plants. Aphid feeding results in the removal of phloem sap and alterations in source-sink patterns, both of which limit plant productivity. In addition, several aphids also vector viral diseases. *Myzus persicae* (Sulzer), commonly known as the green peach aphid (GPA), is a polyphagous insect with a wide-host range that includes the model plant *Arabidopsis thaliana*. Arabidopsis utilizes antibiotic (impact insect fecundity) and antixenotic mechanisms (deter insect settling and limit feeding) to curtail GPA infestation. We have exploited this interaction between *Arabidopsis thaliana* and GPA to characterize the molecular basis of host defense against GPA. Our studies indicate that the *PAD4* (*PHYTOALEXIN-DEFICIENT4*) gene, which encodes a protein that is homologous to eukaryotic α/β fold hydrolases, is an important modulator of Arabidopsis defense against GPA. *PAD4* is required for deterring insect settling on the plant, and for limiting insect feeding from sieve elements and fecundity. In addition, *PAD4* is required for the activation of premature leaf-senescence that is activated in response to the infestation. *PAD4* expression is induced in response to GPA infestation. This increase in *PAD4* expression is modulated by the *TPS11* gene, which encodes an enzyme that synthesizes trehalose, a non-reducing disaccharide. Our studies are suggestive of a regulatory function for *TPS11* and trehalose metabolism in promoting defense against GPA. In addition to modulating *PAD4* expression, the *TPS11* promoted reallocation of carbon into starch at the expense of sucrose, which is the primary plant-derived carbon and energy source for GPA, also contributes to host defense against the insect. Genes similar to *PAD4* and *TPS11* exist in other plants, suggesting that their function in defence against GPA is likely conserved beyond Arabidopsis.

Defense responses against tomato wilt pathogen (*Fusarium oxysporum f. sp. licopersici*) induced with partially identified proteins from two biocontrol Fusaria

Presenting author: **Larisa Shcherbakova** (larisa@vniif.ru)

Authors: Shcherbakova LA[1], Odintsova TI[2], Fravel DR[3], Semina YuV[1], Roberts DP[3]

[1]Russian Research Institute of Phytopathology, RAAS, Moscow region

[2]Vavilov Institute of General Genetics, RAS, Moscow

[3]USDA, ARS, Beltsville Agricultural Research Center, Beltsville, MD

67

Proteins produced by *Fusarium sambucinum* (isolate FS-94) and *F. oxysporum* (isolate CS-20) which are promising as biocontrol agents of Fusarium wilt on tomato were characterized, and defense responses in plants exposed to these proteins were studied.

Protein-containing and non-protein fractions were obtained by separation of high-molecular weight metabolites of FS-94 and CS-20 on Sephacryl S-400. Root immersion of tomato seedlings in the protein-containing fractions before plant inoculation with *F. oxysporum* f. sp. *lycopersici* significantly reduced wilt severity on tomatoes while non-protein fractions were virtually inactive. Enzyme hydrolysis with proteinase K or short-time incubation at 100°C nullified protective effect of treatments with proteins.

The level of *pr-1* expression relative to β-*tubulin* in infected plants which roots were treated with the protein-containing fractions prior to inoculation with the pathogen was much higher than the relative expression level of *pr-1* in water-treated infected seedlings. CS-20 proteins up-regulated *pr-1* more significant as compared to proteins isolated from FS-94. Proteins of FS-94 activated and primed a SA-dependent signaling system of tomato while CS-20 proteins did not affect this signaling pathway. After inoculation by the pathogen, the activity of PAL and chitinase was increased in protein-treated plants compared with control plants. Both FS-94 and CS-20 protein-containing fractions induced a reversible change of extracellular pH in cultured tomato cells and stimulated ROS generation *in planta*.

Protein pattern of the defense response-inducing fractions were analyzed by HPLC, SDS-PAGE (FS-94 and CS-20) and PAGE in a cathodic buffer system (FS-94). The pattern of active FS-94 proteins very differed from the pattern of inactive proteins produced by the same isolate grown under unsuitable conditions. SDS-PAGE showed the presence of a main FS-94 protein of molecular weight (MW) about 50 kDa in the active protein samples and its absence in inactive ones. HPLC followed by MALDI-TOF MS detected CS-20 proteins in the MW range from below 10 to 54 kDa with the main 10 kDa protein. Elimination of 10 kDa from the elicitor fraction abolished its protective effect. Both 50 kDa and 10 kDa were enriched with residues of basic amino acids and produced polypeptides of lower molecular mass in gels with 2-ME, suggesting the presence of interchain S-S bonds. N-terminal sequence of 10 kDa had 62% homology with a hypothetical protein XP390960 from *F. graminearum* and a unique cysteine motif that included 6 Cys residues per 25 amino acids.

SA, JA, ET and ABA all playing roles in signalling of defence response of *Brassica napus* to necrotroph *Sclerotinia sclerotiorum* and biotroph *Pseudomonas syringae* pv. tomato DC3000

Presenting author: **Miroslava Špácová** (mirca.spacova@centrum.cz)

Authors: Špácová M[1,2], Šašek V[1], Valentová O[2], Burketová L[1]

[1]Laboratory of Plant Pathophysiology, Institute of Experimental Botany AS CR, Prague, Czech Republic

[2]Department of Biochemistry and Microbiology, Institute of Chemical Technology, Prague, Czech Republic

68

Signalling events preceding plant defence response to pathogens form complex interconnected signalling network mainly regulated by hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET). Recently, other hormones e.g. abscisic acid (ABA), gibberellins (GA) and auxins, were shown to play a role in defence signalling as well. Increased levels of the signalling hormones trigger massive reprogramming of plant's transcriptome leading to induction of potential defence mechanisms. Generally, defence responses triggered by JA/ET signalling are effective against necrotrophs, while defence dependent on SA signalling is effective against biotrophs. Current view on defence mechanisms is mainly based on studies performed on the model plant *Arabidopsis thaliana.*

In order to reveal the role of each hormone in the signalling events after attack of necrotrophic and biotrophic pathogen, we assessed the interaction of *Brassica napus* with the necrotrophic fungal pathogen *Sclerotinia sclerotiorum*, or biotrophic bacteria *Pseudomonas syringae* pv. *tomato* DC3000. *Brassica napus* was chosen as representative of non-model plant and economically important crop.

First, we followed the level of different plant hormones in leaves after attack with above-mentioned pathogens by LC-MS. Further, we identified a set of genes in *B. napus* homologous to known *Arabidopsis* defence signalling marker genes. Using qPCR we analysed expression of each gene in plants after inoculation with the pathogens. Finally, we treated plants with inducers of SA, ET, JA and ABA signalling pathways and assessed their resistance to the above-mentioned pathogens.

In our experiments, the necrotroph *S. sclerotiorum* induced mostly JA/ET signalling, but besides it also activated SA and ABA signalling. The biotroph *P. syringae* pv. *tomato* DC3000 activated primarily SA dependent signalling, but also JA, ET and ABA signalling were induced. Surprisingly, defence mechanisms induced after activation of SA dependent signalling pathways eliminated both pathogens. In conclusion, our results contribute to the hypothesis that all the signalling pathways can positively contribute to immunity to both biotrophs and necrotrophs.

Response of wheat to infestation by the wheat bulbfly: A proteomics-based approach

Presenting author: **Stylianos Stavroulakis** (stylianos.stavroulakis@ncl.ac.uk)

Authors: Stavroulakis S[1], Bell HA[2], Ferry N[1,3], Gatehouse JA[4], Gatehouse AMR[1]

[1]School of Biology, Newcastle University, Newcastle upon Tyne NE1 7RU, UK
[2]The Food and Environment Research Agency, Sand Hutton, York, UK
[3]School of Environment and Life Science, Salford University, Salford, UK
[4]School of Biological and Biomedical Sciences, University of Durham, Durham, UK

69

Wheat bulb fly (*Delia coarctata*) is an important pest causing 'deadheart' in young wheat (*Triticum aestivum*) plants as a consequence of larval feeding; this can result in significant damage and yield loss. In this study the commercial line Hereward was shown to exhibit the highest levels of resistance to wheat bulb fly in glasshouse trials, whilst Einstein was shown to be highly susceptible. These two varieties were selected for further study to investigate changes in gene expression in response to insect feeding. Approximately 770 protein spots were reproducibly detected in extracts from leaves of wheat seedlings var. Hereward after extraction and 2 DE gel electrophoresis. Twenty protein spots differed significantly ($p<0.05$, ANOVA) between control and infested plants following 2 weeks of larval feeding (i.e. at the stage when 'deadheart' symptoms occur); of these 18 protein spots were up-regulated and 2 down-regulated. Approx 880 protein spots were reproducibly detected in the susceptible var. Einstein, 16 protein spots were significantly ($p<0.05$, ANOVA) differentially expressed, with 8 being up-regulated and 8 being down-regulated. Ten differently expressed protein spots from each of the two varieties were identified by MALDI-TOF mass spectrometry and classified according to function. Whilst the majority of the proteins identified were involved in photosynthesis, some were shown to be involved in signal transduction (Ubiquitin), stress response (1-Cys peroxiredoxin) and plant defence (Inactive disease susceptibility protein LOV1). Additionally, 10 differentially expressed protein spots were also identified by LC/MS; data is currently being analysed.

Functional characterization of the effector protein RxLR19 of the Arabidopsis pathogen *Phytophthora brassicae*

Presenting author: **Michael Stumpe** (michael.stumpe@unifr.ch)

Authors: Stumpe M, Branciard L, Mauch F

Dept. Biology, Plant Biology, University of Fribourg, Switzerland

70

Oomycete pathogens have evolved effector proteins to overcome plant defences. Common features of one group of these effectors are a N-terminal signal peptide followed by the consensus RxLR-EER sequence and a putative defence suppressing functional domain. By screening an EST library of *Phytophthora brassicae*, a pathogen affecting *Arabidopsis thaliana*, we identified a number of RxLR proteins.

Here, we present the functional characterisation of the 166 amino acid long RxLR19 protein that has homologues in *P. infestans* and *P. sojae*. Transgenic plants expressing RxLR19 showed upon infection with *P. brassicae* an increased expression of the salicylic acid (SA) pathway marker gene *PR1* and a reduced expression of the jasmonic acid (JA) marker gene *PDF1.2*.

Yeast-2-hybrid screening revealed a direct interaction of RxLR19 with the 14-3-3 protein AtGRF6 previously described as a positive regulator of RPW8-mediated disease resistance against powdery mildew.

Inoculated *grf6* mutants showed reduced PR1 expression, thus, identifying AtGRF6 as a positive regulator of SA-signalling. Surprisingly, *grf6* plants showed enhanced resistance to *P. brassicae* indicating that GRF6 acts as a negative regulator in defence of Arabidopsis against *P. brassicae*. Our results suggest that RxLR19 indirectly represses JA-signalling by boosting SA-signalling via its positive interaction with AtGRF6.

Chemical compounds used by a parasitic wasp *Aphidius ervi* in discrimination between volatiles from host-infested plants and nonhost-infested plants

Presenting author: **Hiroyuki Takemoto** (takemoto@ecology.kyoto-u.ac.jp)

Authors: Takemoto H[1], Pickett JA[2], Powell W[2], Kainoh Y[3], Takabayashi J[1]

[1]Center for Ecological Research, Kyoto University, Japan
[2]Rothamsted Research, UK
[3]Institute of Agriculture and Forestry, University of Tsukuba, Japan

71

Parasitic wasps use volatile organic compounds (VOCs) emitted by plants infested by their hosts, when searching for their hosts. The quality and/or quantity of those VOCs can be specific according to herbivore species, and this specificity may facilitate the host searching by parasitoids. If so, an intriguing question is how the wasps could respond to such a specific blend of VOCs from host-infested plants.

Our previous report has shown that aphid parasitic wasps, *Aphidius ervi*, discriminate between VOCs from *Acyrthosiphon pisum* (host)-infested plants and VOCs from *Aphis craccivora* (nonhost)-infested plants. We analyzed the chemical composition of the volatile blends from *A. pisum*-infested plants and from *A. craccivora*-infested plants, and we selected 6 host-specific compounds (β-myrcene, *n*-octanal, ±-phellandrene, (*E*)-β-ocimene, γ-terpinene, and (*R*)-linalool) and two nonhost-specific compounds (GLVs; (*Z*)-3-hexen-1-ol and (*Z*)-3-hexen-1-yl acetate) to test their potential effect on the discrimination by wasps between host/nonhost-induced volatiles.

Wasps preferred the blend of the host-specific compounds in all concentrations we tested (5 ng, 1 ng and 0.1 ng (each compound)) In Y-tube olfactometer assays. When these compounds were offered in single, wasps showed preference in three of the compounds (*n*-octanal, (*R*)-α-phellandrene, and (*E*)-β-ocimene) over n-hexane control. Among them, wasps showed the preference for (*R*)-α-phellandrene in the same dose as they did in the blend of 6 compounds (0.1 ng). Wasps preferred *n*-hexane control over (*R*)-linalool which is emitted from *A. craccivora*-infested plants in larger quantity compared to the *A. pisum*-infested plants. Addition of nonhost-specific compounds (mixture of (*Z*)-3-hexen-1-ol and (*Z*)-3-hexen-1-yl acetate) reduced the attractiveness of VOCs from *A. pisum*-infested plants. These data suggest that preference for the specific compounds is involved in the response by wasps to the VOCs from host-infested plants, and that relatively large amounts of GLVs and (*R*)-linalool, which is specific in *A. craccivora*-infested plants compared to *A. pisum*-infested plants, reduce the attractiveness of the host-specific VOCs, such as *n*-octanal, α-phellandrene, and (*E*)-β-ocimene.

A jasmonic acid-independent COI1 function mediates partial resistance of *Arabidopsis thaliana* against the vascular pathogen *Verticillium longisporum*

Presenting author: **Corinna Thurow** (cthurow@gwdg.de)

Authors: Ralhan A, Thurow C, Gatz C

Albrecht-von-Haller-Institute for Plant Sciences, Department of Molecular Biology and Physiology of Plants, Georg-August-University Goettingen

72

Plant jasmonates (JAs) and their receptor CORONATINE INSENSITIVE1 (COI1) play a central role in defense responses against necrotrophic pathogens. Consequently, JA signaling (*coi1*) and biosynthesis (*dde2*) mutants display enhanced disease susceptibility to this type of pathogens. In contrast, we report here that disease symptoms like stunted growth and early senescence caused by the vascular pathogen *Verticillium longisporum* appear with a reduced frequency in *coi1* plants. The healthy looking phenotype correlated with reduced fungal colonization and a lower percentage of *coi1* plants allowing microsclerotia formation. Contrary to the expectation that the hormone receptor mutant *coi1* should display the same phenotype as the corresponding hormone biosynthesis mutant *dde2*, *dde2* plants allowed wild-type-like fungal propagation and displayed typical *V. longisporum*-induced disease symptoms. Marker genes of the JA- or JA/ethylene defense pathway were elicited in wild-type but not in *dde2* plants indicating the absence of fungal compounds that would activate the known COI1-dependent processes.

Microarray analysis comparing uninfected and infected plants at 15 days post infection revealed that 1936 genes were differentially regulated. Of these, only 112 genes exhibit an expression pattern which correlates with the pathophenotype of wild-type, *dde2*, and *coi1* plants, respectively. The majority of these 112 genes are not inducible by JA, supporting the notion that other signals lead to the activation of this specific set of COI1-dependent genes that are expressed in the *dde2* mutant. In conclusion, we have found an apparently JA-independent function of COI1 which is required for successful plant colonization by *V. longisporum* in the absence of detectable amounts of oxylipins or oxylipin mimics.

The effect of *Tetranychus urtice* Koch on total phenol content and activity of poliphenoloxidase and peroxidase in cucumber plants treated with selected resistance inducers and biostimulators

Presenting author: **Anna Tomczyk** (anna_tomczyk@sggw.pl)

Authors: Tomczyk A

Department of Applied Entomology, Warsaw Agricultural University, Nowoursynowska 166, 02-787 Warsaw, Poland

73

The aim of the study was to evaluate if the application of resistance inducers: Milsana reagent (extract of *Reynoutria sachalinensis*) and plant growth promoting rhizobacteria (*Pseudomonas fluorescens*) as well as biostimulators: Asahi SL and Siapton 10 L on the plants infested by spider mites can change the reaction of injured plants to the spider mites as well as development of pest population.

Two separate experiments were conducted on cucumber cv. Aramis growing under glasshouse conditions and treated with resistance inducers or biostimulators. Total phenol content as well as activity of poliphenol oxidase and peroxidase was measured in infested plants after 6-7 weeks of spider mite feeding. Spider mite population was simultaneously monitored on plants treated and untreated with resistance inducers or biostimulators.

The content of phenols in the leaves of the plants treated with resistance inducers showed the tendency to minor increase, however, not in the plants injured by mites. The treatment by biostimulators decreased phenol concentration in both untreated and treated leaves.

Little changes in poliphenol oxidase activity were observed in the leaves of plants treated with the resistance inducers, as compared to control. Spider mite feeding caused an increase in poliphenol oxidase activity from 10 % till 37%, however, the highest increase was observed in the leaves of plants untreated with resistance inducers. Infested plants treated with Siapton SL had the highest activity of poliphenol oxidase as compared to control plants

Activity of peroxidase was increased after the application of both resistance inducers on cucumber plants. The effect of Milsana was more evident as compared to the treatment with bacteria. High increase in peroxidase activity was found in all plants infested by spider mites. However, application of resistance inducers caused the decline in the intensity of this reaction in mite-infested leaves. An application of both biostimulators on cucumber plants caused significant increase in peroxidase activity in both uninfested and mite-infested plants.

The spider mites populations in all plants treated with resistance inducers and biostimulators were lower as compared to control.

Microorgans in herbivorous spider mites (*Tetranychus urticae*) regulate ecological interactions with lima bean plant

Presenting author: **Hirokazu Ueda** (no_hi-ueda@nara.kindai.ac.jp)

Authors: Ueda H[1,2], Ozawa R[2], Takabayashi J[2] Maffei ME[3], Matsuda K[2]

[1]Faculty of Agriculture, Kinki University, Japan

[2]Center for Ecological Research, Kyoto University, Japan

[3]Department of Plant Biology and Centre of Excellence CEBIOVEM, University of Turin, Italy

74

In response to herbivores attack, plants defense against herbivores are not only physical and chemical barriers that directly aim to harm their attackers, plants also employ strategies of indirect defense. One form of indirect defense in plants is to attract predators and parasitoids by emitting specific blends of volatiles to defense from herbivores' attack. These volatiles are referred to as herbivore-induced plant volatiles (HIPVs), which are released specifically in response to herbivore attack. We hypothesized that microorganisms in spider mites (*Tetranychus urticae*) could regulate the induction of HIPVs, because spider mites were found to induce accumulation of salicylic acid (SA) as well as expression of SA-inducible genes in lima bean plants. Thus microorganisms in spider mites were postulated to be involved in the production of HIPVs. Two types of microbes may exist in spider mites. Namely, one type is microbe present in the digestive tract or surface (exo-microbe). Another type is intercellular microbe (endo-microbe). We prepared three types of microbe-free spider mites that are named -exomicrobe (-Exo), -endomicrobe (-Endo), -all microbe (-All) and normal one. We analyzed the amount of HIPVs, levels of SA, and expression of several defense genes and observed Ca^{2+} and H_2O_2 accumulation in lima bean (*Phaseolus limensis*) leaves. In these respects, significant differences were observed between normal and each sterilized spider mites, suggesting that each two types of microorganisms in spider mites are involved, at least in part, in the induction of the specific blend of HIPVs.

Discovery of WRKY transcription factors involved in activation of SA biosynthesis genes; a bioinformatics and molecular biology approach

Presenting author: **Marcel Van Verk** (m.c.vanverk@uu.nl)

Authors: Van Verk MC[1,2], Bol JF[1], Linthorst HJM[1]

[1]Institute of Biology, Department of Plant Cell Physiology, Leiden University, the Netherlands

[2]Plant Microbe Interactions, Department of Biology, Utrecht University, the Netherlands

75

In order to comprehend the mechanisms of induced plant defense, knowledge of the regulation of the biosynthesis of the defense hormones salicylic acid (SA), jasmonic acid (JA) and ethylene (ET) is essential. Potentially, many transcription factors could be involved, but identifying them is a difficult endeavour. Using 372 publicly available microarray data sets, a network was constructed in which Arabidopsis genes known to be involved in SA, JA and ET signaling pathways together with over 1400 transcription factors genes were assayed for co-expression. Many obtained connections between transcription factors and genes involved in the signaling pathways were previously reported in literature to be relevant for stress responses and that fit current models of stress gene regulation, indicating the high potential of our approach. In addition, the derived network suggested new candidate genes and associations that are potentially interesting for future research to further unravel their involvement in responses to stress. Two of those candidate genes, *WRKY28* and *WRKY46*, were identified as possible regulators of *ICS1* and *PBS3* that are involved in SA biosynthesis. Expression studies with *ICS1 promoter::*β*-glucuronidase (GUS)* genes in *Arabidopsis thaliana* protoplasts cotransfected with *35S::WRKY28* showed that over expression of WRKY28 resulted in a strong increase in GUS expression. Moreover, qRT-PCR analyses indicated that the endogenous *ICS1* and *PBS3* genes were highly expressed in protoplasts overexpressing WRKY28 or WRKY46, respectively. Electrophoretic mobility shift assays indentified potential WRKY28 binding sites in the ICS1 promoter, positioned -445 and -460 base pairs upstream of the transcription start site. Mutation of these sites in protoplast transactivation assays showed that these binding sites are functionally important for activation of the *ICS1* promoter. Chromatin immunoprecipitation assays with haemagglutinin-epitope-tagged WRKY28 showed that the region of the *ICS1* promoter containing the binding sites at -445 and -460 was highly enriched in the immunoprecipitated DNA. The results obtained here confirm results from our multiple microarray co-expression analyses indicating that WRKY28 and WRKY46 are transcriptional activators of *ICS1* and *PBS3*, respectively, and support this *in silico* screening as a powerful tool for identifying new components of stress signaling pathways.

Bacterially induced systemic acquired resistance in barley?

Presenting author: **Corina Vlot** (corina.vlot@helmholtz-muenchen.de)

Authors: Vlot AC, Wenig M, Knappe C

Helmholtz Zentrum Muenchen, Institute of Biochemical Plant Pathology, Neuherberg, Germany

76

Until now, it is unclear if systemic acquired resistance (SAR) exists in monocotyledonous plant species and/or how it compares to well-established SAR systems in dicotyledonous plants. SA appears to play a role in disease resistance in, for instance, rice. In addition, SA signalling partners such as NPR1 are conserved in various monocotyledonous plants. While Arabidopsis *NPR1* can protect wheat from disease, *NPR1* from rice is functional in Arabidopsis. We investigated the possibility to induce SAR in barley in order to generate a monocotyledonous SAR pathosystem to test possible protection of cereals via SAR/priming. Infection of the first leaf of 4-week-old barley plants with either *P. syringae* pathovar *japonica* or *Xanthomonas translucens* significantly enhanced resistance in the systemic tissue against *X. translucens*. *P. syringae* grew, but only to limited levels in the inoculated leaf and caused brown spots reminiscent of HR lesions, whereas *X. translucens* seemed virulent, growing to high levels within the leaves causing severe yellowing and eventually death. A primary *X. translucens* infection invariably induced a better protection against subsequent attack than did *P. syringae*. Both primary inoculi caused increased accumulation of SA and glucosylated SA in the infected leaf, but not systemically. Therefore, we are now performing transcriptional analyses in the systemic tissue to investigate which genes are induced and/or repressed during systemic resistance induction in barley. The results of these analyses will be discussed.

The impact of hormonal crosstalk on resistance of plants to multi-species attack

Presenting author: **Irene A. Vos** (i.a.vos@uu.nl)

Authors: Vos IA, Pieterse CMJ and Van Wees SCM

Plant-Microbe Interactions, Department of Biology, Utrecht University, the Netherlands

77

In nature plants are threatened by multiple pathogens and herbivorous insects. Plants have evolved a complex immune system to protect themselves against different attacking organisms. The underlying plant signaling network is controlled by diverse phytohormones of which salicylic acid (SA) and jasmonates (JA) emerged as core players. Their signaling pathways cross-communicate, providing the plant with a highly flexible regulatory capacity to finely tune its immune response against the attacker[1,2]. We aim to investigate the role of defense signaling crosstalk in resistance and in defense gene activation when the plant is attacked simultaneously by multiple harmful organisms.

The antagonistic interaction between the SA and JA response pathways is one of the best studied examples of signaling crosstalk. Pharmacological experiments with *Arabidopsis thaliana* (Arabidopsis) revealed that JA-responsive marker genes, such as *PDF1.2*, *VSP2* and *LOX2* are highly sensitive to suppression by exogenous application of SA. Defenses dependent on SA are generally effective against biotrophic pathogens, while JA-regulated defenses are generally effective against necrotrophic pathogens and herbivorous insects. Research on the role of SA/JA pathway crosstalk in the regulation of plant immunity during interactions with *multiple* attackers is still in its infancy. We use SA- and JA-sensitive GUS reporter lines to determine which signaling pathway is prioritized when Arabidopsis is under attack by combinations of the biotrophic pathogen *Hyaloperonospora arabidopsidis*, the necrotrophic pathogen *Botrytis cinerea,* and the herbivorous insect *Pieris rapae*. In parallel, we monitor gene expression induced by exogenous application of combinations of SA, JA and the other phytohormones ethylene (ET) and abscisic acid (ABA), which are known modulators of SA and JA signaling. These transcriptome data will be correlated with studies on disease resistance against multi-species attack to verify the impact of SA/JA crosstalk on plant performance under natural conditions with multiple attackers.

Pieterse, C.M.J., Leon-Reyes, A., Van der Ent, S. & Van Wees, S.C.M. (2009). Networking by small-molecule hormones in plant immunity. *Nat. Chem. Biol*. 5, 308-316.

Verhage, A., Van Wees, S.C.M. & Pieterse, C.M.J. (2010). Plant immunity: It's the hormones talking, but what do they say? *Plant Physiol.* 154, 536-540.

Transcript profiles in different sugar beet genotypes uncover timing and strength of defense reactions to *Cercospora beticola*-infection

Presenting author: **Fridtjof Weltmeier** (f.weltmeier@kws.com)

Authors: Weltmeier F[1], Mäser A[1], Menze A[2], Brieß W[1], Klages S[1], Werner N[1], Kurrasch J[1], Holtschulte B[2], Hennig S[3], Schad M[3], Nehls R[1], Stahl DJ[1]

[1]Planta GmbH, Grimsehlstraße 31, D-37555 Einbeck, Germany
[2]KWS SAAT AG, Grimsehlstraße 31, D-37555 Einbeck, Germany
[3]ImaGenes GmbH, Robert-Rössle-Straße 10, D-13125 Berlin, Germany

78

Plant diseases caused by fungal infections result in serious yield and quality losses of crops. Leaf spot disease, caused by the filamentous fungus *C. beticola*, is considered to be the most destructive disease of sugarbeet worldwide. Resistance to *C. beticola* in sugarbeet has been described as quantitatively inherited and rate limiting with respect to disease development, except for sugarbeet varieties possessing the dominant Cb resistance gene. In these varieties, the *C. beticola* field isolate C2 can induce a low-virulence 'fleck' reaction, leading to resistance. However, the molecular mechanisms leading to monogenic or polygenic resistance have not been well studied.

During the last decade, microarrays have turned out to be a powerful tool to probe the expression level of thousands of genes simultaneously. As biological processes are reflected to a high degree by changes in the transcript abundance of involved genes, expression profiling facilitates a better understanding of the underlying molecular mechanisms. To make the advantages of microarray-based genome-wide expression profiling available for sugar beet, we developed a 4x44K oligonucleotide-based sugarbeet microarray with the ability to detect the transcripts of 17.277 genes. We applied this microarray to analyze the transcriptional changes underlying monogenic and polygenic resistance of sugarbeet to *C. beticola*.

A similar set of genes was induced in all types of interaction, but with striking differences in timing. While the monogenic resistant interaction displayed strong defense responses as soon as one day post inoculation (1 dpi), in the partial resistant and susceptible interactions defense responses were delayed to a late phase (15 dpi) of the infection cycle. In the early response the group of genes with the highest induction levels was dominated by genes involved in signaling, while in the late response highest induction levels were observed for PR genes and genes involved in lignin and alkaloid biosynthesis.

Further, the defense response of the partial resistant genotype was stronger compared to the defense response of the susceptible genotype, and the partial resistant genotype expressed pathogen related transcripts that the susceptible genotype lacked. These results indicate that resistance was achieved by the ability to mount an early defense response, and partial resistance was determined by additional defense and signaling transcripts that allowed more effective defense in the late phase of the infection cycle.

Effect of heat shock treatment in inducing melon plant resistance against *Botrytis cinerea*

Presenting author: **Ani Widiastuti** (aniwidiastuti@ugm.ac.id)

Authors: Widiastuti A[1,4], Yoshino M[2], Zhou S[2], Odani H[2], Hasegawa M[3], Nitta Y[3], Sato T[2]

[1]United Graduate School of Agricultural Science, Tokyo University of Agriculture and Technology, 3-5-8 Saiwai, Fuchu, Tokyo, 183-8509, Japan
[2]Field Science Center, College of Agriculture, Ibaraki University, Ami 4668-1, Ami, Inashiki, Ibaraki, 300-0331, Japan
[3]College of Agriculture, Ibaraki University, 3-21-1 Chuo, Ami, Inashiki, Ibaraki, 300-0393, Japan
[4]Faculty of Agriculture, Gadjah Mada University, Yogyakarta, 55281, Indonesia

79

Heat stress is a major factor limiting the productivity and adaptation of crops production, especially when temperature extremes coincide with critical stages of plant development. The rate of temperature change and the duration and degree of high temperatures all contribute to the intensity of heat stress. Some experiments demonstrated that heat stress in certain degree can be utilized for increasing plant defense against pathogens infection. Therefore, in this study we searched combination of high temperature of water and short dipping duration as heat shock treatment and its capability to reduce gray mold disease in melon seedlings.

Our research found that at 50 ^{o}C 20 s was the most effective heat shock treatment to reduce the infection of *Botrytis cinerea*, causal agents of gray mold disease, in melon seedlings. Soon after that heat shock treatment, plants demonstrated slight wilting but recovered within one day after treatment, indicating that the seedlings could tolerate the heat stress. Heat shock at that point did not directly inhibit mycelia growth of pathogen assuming that resistance enhanced mechanism on plants possibly involved after heat shock treatment rather than direct inhibiting against the pathogen growth. Heat shock treatment also increased expression level of some genes related to plant resistance and priming of Peroxidase (POX) gene was assumed as one mechanism that possibly involved in the heat shocked plant-pathogen interaction of melon plants.

Barley targets of YxC-effectors from the powdery mildew fungus

Presenting author: **Wen-Jing Zhang** (wzg@life.ku.dk)

Authors: Zhang W-J, Pedersen C, Thordal-Christensen H

Department of Agriculture and Ecology, Faculty of Life Sciences, University of Copenhagen, Denmark

80

Biotrophic pathogenic fungi (e.g. powdery mildew fungi) rely on specialized organs (haustoria), which they make inside the plant cells. A specialized plant membrane is generated around the haustorium, which thereby become separated from the plant cytosol. By an unknown mechanism, YxC-effector proteins produced by the haustorium of the barley powdery mildew fungus (*Blumeria graminis* f.sp. *hordei*, *Bgh*) (Godfrey et al., 2010) are transferred to the plant cell in order to re-direct nutrient or suppress the plant defense mechanisms.

Here we aim to identify barley proteins that interact with three highly expressed *Bgh* YxC-effector candidates by using yeast 2-hybrid. Interactor candidates found this way will be confirmed by Bimolecular Fluorescence Complementation (BiFC) analyses. Such interactors are expected to include proteins of different functional categories. They can be proteins that bind the YxC motif, which is common in these effector candidates. They can also include defence signalling proteins or proteins mobilizing nutrients to the fungus.

Our yeast 2-hybrid has so far suggested interactors for two of the effector candidates. These suggested interactors appear to be related to disease resistance, since silencing of them in barley results in increased susceptibility to *Bgh*. The results hint that the two effectors are involved in suppressing the plant defence mechanisms. The role of the identified target proteins will be further analysed by biochemical, molecular, cellular and genetic methods.

Godfrey, D., Böhlenius, D., Pedersen, C., Zhang, Z., Emmersen, J., Thordal-Christensen, H. Powdery mildew fungal effector candidates share N-terminal Y/F/WxC- motif (2010) BMC Genomics 11: 317-330

Proteomic characterization of grapevine resistance against downy mildew activated by *Trichoderma harzianum* T39

Presenting author: **Maria Cristina Palmieri** (mariacristina.palmieri@iasma.it)

Authors: Palmieri MC[1], Perazzolli M[1], Matafora V[2], Bachi A[2], Pertot I[1]

[1]IASMA Research and Innovation Centre, Fondazione Edmund Mach, via E. Mach 1, San Michele all'Adige 38010, Trento, Italy
[2]Biological Mass Spectrometry Unit DIBIT, San Raffaele Scientific Institute, via Olgettina 58, Milano 20132, Italy

81

The host-specific oomycete *Plasmopara viticola* is the causal agent of downy mildew, one of the most important grapevine diseases. In recent years, several molecules able to induce a plant-mediated resistance against *P. viticola* have been described. Some inducers, i.e. benzothiaziadole (BTH), activate a direct resistance involving constitutive barriers and local and systemic defences, with a high metabolic cost for the plant. On the other hand *Trichoderma harzianum* T39, a beneficial microorganism, primes for defence against grapevine downy mildew. This mechanism presents advantages in terms of energy costs for the plant, because defence responses are expressed upon pathogen attack and, therefore, only when they are really needed.

The present study aims to understand cellular processes involved in reaction to *P. viticola* inoculation in plants pre-treated with the biocontrol agent *T. harzianum* T39 in comparison to the water-treated and BTH-treated ones. By monitoring the kinetic of intercellular colonization of *P. viticola*, callose and lignin deposition in grapevine cell wall and the production of reactive oxygen species, we demonstrated a priming mechanism for the post-invasion reactions in T39- and BTH-treated plants with difference in timing and magnitude of responses.

Moreover, to explore the processes involved in grapevine self-defence induced by *T. harzianum* T39 before and after pathogen inoculation, a proteomic approach was used following an 8-plex differential mass tags iTRAQ protocol. Among proteins identified and quantified by LC-MS-MS (more than 900), 89 were significantly modulated 1 day post *P. viticola* inoculation in water-treated plants. Eighty-three proteins were directly modulated by T39 prior pathogen infection and 104 in T39-treated plants at 1 day post *P. viticola* inoculation. A general re-direction of primary and secondary metabolism, together with a reaction involving early steps of recognition (receptors and downstream signalling molecules) were induced by *P. viticola* within the first day of infection in water-treated plants. On the contrary a general number of proteins including hormones signalling, pathogenesis related proteins and oxidative stress-related proteins, were induced or primed for induction upon pathogen inoculation in T39-treated plants.

All these results offer a greater understand of the mechanisms underlying the grapevine T39-induced resistance and illustrate the priming effect of T39 on both defence-related proteins abundance and constitutive barriers formation. Further research will be focused on a detailed functional characterization of potential elicitors of grapevine defence mechanism.

Index: talks

V.

Index: posters

VI.

Author index

VII.

Participant list

VIII.

A

B

Participant	Inst. affiliaton	Country	Email
ADEVI SENAM MIREILLE, Broohm	Institut privé laïc de recherche et de la formation professionnelle «EUREKA»	France	eurekainstitut@yahoo.fr
ADRIAN, Marielle	INRA 1088 Plante Microbe Environnement	France	Marielle.Adrian@u-bourgogne.fr
AHMAD, Shakoor	Rothamsted Research	United Kingdom	shakoor.ahmad@bbsrc.ac.uk
ALEXANDERSSON, Erik	Swedish University of Agricultural Sciences, Dept. of Plant Protection Biology	Sweden	erik.alexandersson@slu.se
ARIMURA, Gen-ichiro	Center for Ecological Research, Kyoto University	Japan	garimura@ecology.kyoto-u.ac.jp
AVER'YANOV, Andrey	Research Institute of Phytopathology, B. Vyazemy, Moscow region	Russia	aaveryanov@post.ru
AYACHI, Sanaa	University of Neuchâtel	Switzerland	sanaa.ayachi@unine.ch
BALMER, Dirk	University of Neuchâtel	Switzerland	dirk.balmer@unine.ch
BARDIN, Marc	INRA, UR407 Pathologie Végétale, Montfavet	France	marc.bardin@avignon.inra.fr
BARTELS, Sebastian	Botanical Institute, University Basel	Switzerland	sebastian.bartels@unibas.ch
BEJAI, Sarosh	Swedish University of Agricultural Sciences, Uppsala	Sweden	Sarosh.Bejai@slu.se
BEN ELOUJA EP HAJRI, Emna	University of Fribourg	Switzerland	emna.beneloujaephajri@unifr.ch
BERGER, Valentine	Université de Neuchâtel	Switzerland	valentine.berger@unine.ch
BERNIER, David	Bayer S.A.S. - Bayer CropScience	France	david.bernier@bayer.com
BESTE, Lisa	Stockholm University	Sweden	lisa.beste@botan.su.se
BINDA, Matteo	University of Fribourg	Switzerland	matteo.binda@unifr.ch
BOBILLIER, Christiane	Université de Neuchâtel	Switzerland	christiane.bobillier@unine.ch
BRANCIARD, Lorelise	University of Fribourg	Switzerland	lorelise.branciard@unifr.ch
BROTMAN, Yariv	Max-Planck-Institute of Molecular Plant Physiology, Potsdam-Golm	Germany	brotman@mpimp-golm.mpg.de
BRÜSSOW, Friederike	University of Lausanne	Switzerland	friederike.bruessow@unil.ch
BURKETOVA, Lenka	Institute of Experimental Botany AS CR, Prague	Czech Republic	burketova@ueb.cas.cz

Participant	Inst. affiliaton	Country	Email
BURZI, Pier Luigi	Agricultural Research Council, Industrial Crop Research Centre, Bologna	Italy	pierluigi.burzi@entecra.it
C			
CAMMUE, Bruno	Katholieke Universiteit Leuven	Belgium	bruno.cammue@biw.kuleuven.be
CARUSO, Carla	Dept. Agrobiology and Agrochemistry, Tuscia University	Italy	caruso@unitus.it
CHLEBNY, Igor	Université de Neuchâtel / NCCR Plant Survival	Switzerland	igor.chlebny@unine.ch
COLAS, Seven	Université de Reims Champagne Ardenne, Laboratoire SDRP, Reims cedex 2	France	steven.colas@etudiant.univ-reims.fr
CONRATH, Uwe	Plant Physiology Department, RWTH Aachen University	Germany	uwe.conrath@bio3.rwth-aachen.de
COOLEN, Silvia	Plant-Microbe Interactions, Utrecht University	The Netherlands	s.coolen@uu.nl
CORDELIER, Sylvain	Université de Reims Champagne Ardenne, Laboratoire SDRP, Reims cedex 2	France	sylvain.cordelier@univ-reims.fr
CORDIER, Mélanie	University of Neuchâtel	Switzerland	melanie.cordier@unine.ch
CORIO-COSTET, Marie-France	INRA, UMR Santé et Agroécologie du Vignoble (SAVE-1065), ISVV	France	coriocos@bordeaux.inra.fr
D			
DA ROCHA, Andrea	Universal Leaf Tabacos	Brazil	arocha@unileaf.com.br
DAIRE, Xavier	UMR Plante-Microbe-Environnement INRA 1088/ CNRS 5184/ Université de Bourgogne	France	xavier.daire@dijon.inra.fr
DE LANGE, Elvira	Institute of Biology, University of Neuchatel	Switzerland	elvira.delange@unine.ch
DELAUNOIS, Bertrand	Université de Reims-Champagne Ardenne, URVVCSE, Reims cedex 2	France	bertrand.delaunois@univ-reims.fr
DIAZ, Jose	Universidade da Coruña, Depto. de Bioloxia Animal, Bioloxia Vexetal e Ecoloxia	Spain	josefv@udc.es
DINH, Kim Phuong	Institute of Chemical Technology Prague	Czech Republic	dinhkimp@vscht.cz
DJUPEDAL, Ingela	Department of Biology, ETH Zürich	Switzerland	ingelad@ethz.ch
DRIOUICH, Azedine	University of Rouen	France	Azeddine.Driouich@univ-rouen.fr
DRÖGE-LASER, Wolfgang	Julius-von-Sachs-Insitut, University of Würzburg	Germany	wolfgang.droege-laser@uni-wuerzburg.de
DUFOUR, Marie-Cécile	INRA, UMR Santé et Agroécologie du Vignoble (SAVE-1065), ISVV	France	dufour@bordeaux.inra.fr
E			
ELLENDORFF, Ursula	Rijk Zwaan	The Netherlands	m.weijers@rijkzwaan.nl

Participant	Inst. affiliaton	Country	Email
F			
FERNÁNDEZ LÓPEZ, Iván	Estación Experimental del Zaidín, CSIC, Granada	Spain	ivan.fernandez@eez.csic.es
FLORS, Victor	Universitat Jaume I, Castellón	Spain	flors@uji.es
G			
GAILLARD, Mickael	Université de Neuchâtel	Switzerland	mickael.gaillard@unine.ch
GALLETTI, Stefania	Agricultural Research Council, Industrial Crop Research Centre, Bologna	Italy	stefania.galletti@entecra.it
GARCIA-RAMIREZ, Juan M	Estacion Experimental del Zaidin, CSIC, Granada	Spain	jmgarcia@eez.csic.es
GATZ, Christiane	Albrecht-von-Haller-Institute for Plant Sciences, Georg-August-University Goettingen	Germany	cgatz@uni-goettingen.de
GERARD, Clémentine	Université de Reims Champagne Ardenne, Laboratoire SDRP, Reims cedex 2	France	clementine.gerard@etudiant.univ-reims.fr
GLAUSER, Gaetan	FARCE, Institute of Biology, University of Neuchâtel	Switzerland	gaetan.glauser@unine.ch
GONZALEZ-TEUBER, Marcia	Max Planck Institute for Chemical Ecology, Jena	Germany	mgonzalez-teuber@ice.mpg.de
GOUINGUENÉ, Sandrine	University of Neuchâtel	Switzerland	sandrine.gouinguene@unine.ch
H			
HALLIER, Sonia	Vegenov-BBV, St Pol de Léon	France	hallier@vegenov.com
HAMON, Céline	Vegenov-BBV, St Pol de Léon	France	hamon@vegenov.com
HAUSE, Bettina	Leibniz Institute of Plant Biochemistry, Halle/Saale	Germany	bhause@ipb-halle.de
HELD, Matthias	University of Neuchâtel	Switzerland	matthias.held@unine.ch
HENNIG, Jacek	Institute of Biochemistry and Biophysics, Warsaw	Poland	jacekh@ibb.waw.pl
HUSSAIN, Rana Muhammad Fraz	Department of Plant Cell Physiology, Institute of Biology, Leiden University	The Netherlands	r.m.f.hussain@biology.leidenuniv.nl
J			
JAKAB, Gabor	Department of Plant Physiology, Faculty of Sciences, University of Pecs	Hungary	jakab@gamma.ttk.pte.hu
JANDA, Martin	Institute of Chemical Technology Prague	Czech Republic	martin.janda@vscht.cz
JAUSLIN, Carol	NCCR Plant Survival	Switzerland	carol.jauslin@unine.ch
JOHANSSON, Anna	Uppsala Biocenter, Swedish University of Agricultural Sciences	Sweden	anna.h.johansson@slu.se
JONSSON, Lisbeth	The Department of Botany, Stockholm University	Sweden	lisbeth.jonsson@botan.su.se

Participant	Inst. affiliaton	Country	Email
JORDÁ, Lucía	CBGP (UPM-INIA), Universidad Politécnica Madrid	Spain	lucia.jorda@upm.es
JUHÁSZ, Csilla	Plant Protection Institute, Hungarian Academy of Sciences	Hungary	juhasz.csilla@nki.hu
JUNG, Sabine	Estación Experimental del Zaidín, (CSIC), Granada	Spain	sabine.jung@eez.csic.es
K			
KATAGIRI, Fumiaki	Department of Plant Biology, University of Minnesota, MN	USA	katagiri@umn.edu
KESSLER, Andre	Cornell University, Ithaca NY	USA	ak357@cornell.edu
KIELKIEWICZ, Malgorzata	Warsaw University of Life Sciences - SGGW, Department of Applied Entomology	Poland	malgorzata_kielkiewicz@sggw.pl
KIRÁLY, Lóránt	Plant Protection Institute, Hungarian Academy of Sciences	Hungary	lkir@nki.hu
KLESSIG, Daniel	Boyce Thompson Institute for Plant Research, Ithaca NY	USA	dfk8@cornell.edu
KNOBLOCH, Thomas	Bayer Cropscience, Lyon Cedex 09	France	thomas.knobloch@bayer.com
KOGEL, Karl-Heinz	Justus Liebig University Giessen	Germany	karl-heinz.kogel@agrar.uni-giessen.de
KONING, Sonja	Enza Zaden R&D B.V.	The Netherlands	receptie_oosterdijkgebouw@enzazaden.nl
KRZYMOWSKA, Magdalena	Institute of Biochemistry and Biophysics, Warsaw	Poland	krzyma@ibb.waw.pl
L			
L'HARIDON, Floriane	University of Fribourg, department of plant biology	Switzerland	floriane.lharidon@unifr.ch
LANGUETIN, Didier	University of Neuchâtel	Switzerland	didier.languetin@unine.ch
LAPIKOVA, Vera	Research Institute of Phytopathology, B. Vyazemy, Moscow region	Russia	aaveryanov@post.ru
LATORSE, Marie-Pascale	Bayer Cropscience, Lyon Cedex 09	France	marie-pascale.latorse@bayer.com
LENK, Andrea	Dept. Agriculture and Ecology, University of Copenhagen	Denmark	anle@life.ku.dk
LEON-REYES, Antonio	Universidad San Francisco de Quito	Ecuador	aleon@usfq.edu.ec
LLORENS, Eugenio	Universitat Jaume I, Castellón	Spain	ellorens@camn.uji.es
LOPEZ-RAEZ, Juan Antonio	Estación Experimental del Zaidín, (CSIC), Granada	Spain	juan.lopezraez@eez.csic.es
LUNA DIEZ, Estrella	Rothamsted Research	United Kingdom	estrella.luna-diez@bbsrc.ac.uk

Participant	Inst. affiliaton	Country	Email
M			
MAFFEI, Massimo	Department of Plant Biology, Plant Physiology Unit, University of Turin	Italy	massimo.maffei@unito.it
MARIUTTO, Martin	Laboratory of Plant Molecular Biology and Biotechnology, University of Liège	Belgium	martin.mariutto@doct.ulg.ac.be
MARTINEC, Jan	Institute of Experimental Botany ASCR	Czech Republic	martinec@ueb.cas.cz
MARTINEZ MEDINA, Ainhoa	Estación Experimental del Zaidín, CSIC, Granada	Spain	ammedina@cebas.csic.es
MASCHER, Fabio	Agroscope Changins-Wädenswil, Nyon	Switzerland	fabio.mascher@acw.admin.ch
MASROOR, Ashir	University of Hohenheimm Stuttgart	Germany	ashermasroor@hotmail.com
MAUCH, Felix	University of Fribourg	Switzerland	felix.mauch@unifr.ch
MAUCH-MANI, Brigitte	University of Neuchâtel	Switzerland	brigitte.mauch@unine.ch
MAZEYRAT GOURBEYRE, Florence	Université de Reims Champagne Ardenne Laboratoire SDRP, Reims cedex 2	France	florence.gourbeyre@univ-reims.fr
MEIJER, Johan	Swedish University of Agricultural Sciences, Uppsala	Sweden	johan.meijer@slu.se
MENTZEL, Tobias	BASF Plant Science Company GmbH	Germany	tobias.mentzel@basf.com
MINDJE ABESSOLO EPSE BETELEGNIE, Odette Marie Solange	Food for All Foundation	Cameroon	ffa_foundation@yahoo.fr
MIRANDA, Livia Atauri	University of Neuchâtel	Switzerland	livia.atauri@unine.ch
MOLINA, Antonio	CBGP (UPM-INIA), Universidad Politécnica Madrid	Spain	antonio.molina@upm.es
MØRCH, Sara Melhedegård	Dept. Agriculture and Ecology, University of Copenhagen	Denmark	saram@life.ku.dk
MORIN, Shai	Hebrew University of Jerusalem	Israel	morin@agri.huji.ac.il
MOSER, Tina	Julius-Kühn-Institut, Institut für Rebenzüchtung Geilweilerhof, Siebeldingen	Germany	tina.moser@jki.bund.de
MUGO, Cynthia	Yamaguchi University	Japan	cynthmugo@yahoo.ca
MUZAMMIL, Saima	Université de Toulouse, LGC UMR 5503, Castanet-Tolosan Cedex 1	France	saimamuzammil83@yahoo.com
N			
NAVAROVA, Hana	Heinrich-Heine University, Düsseldorf	Germany	hana.navarova@uni-duesseldorf.de
NEELEY, David	Universität Hohenheim, Stuttgart	Germany	neeley@gmx.de

Participant	Inst. affiliaton	Country	Email
NICOT, Philippe	INRA - UR407 Pathologie végétale, Montfavet Cedex	France	philippe.nicot@avignon.inra.fr
ONKOKESUNG, Nawaporn	Laboratory of Entomology, Wageningen University	The Netherlands	nawaporn.onkokesung@wur.nl
ORLIK, Marc	Julius Kühn-Institut, Institute for Biological Control, Darmstadt	Germany	marc.orlik@jki.bund.de
PALMIERI, M. Cristina	Fondazione Edmund Mach, IASMA Research and Innovation Centre	Italy	mariacristina.palmieri@iasma.it
PASTOR, Victoria	Universitat Jaume I, Castellón	Spain	victoria.bitacora@gmail.com
PEDERSEN, Carsten	Dept. Agriculture and Ecology, University of Copenhagen	Denmark	cpr@life.ku.dk
PELISSIER, Bernard	Bayer Cropscience, Lyon Cedex 09	France	bernard.pelissier@bayer.com
PERAZZOLLI, Michele	Fondazione Edmund Mach, IASMA Research and Innovation Centre	Italy	michele.perazzolli@iasma.it
PERTOT, Iliaria	Fondazione Edmund Mach, IASMA Research and Innovation Centre	Italy	ilaria.pertot@iasma.it
PFITZNER, Ursula	Universität Hohenheim, Stuttgart	Germany	pfitzner@uni-hohenheim.de
PICOT, Adeline	Vegenov-BBV, St Pol de Léon	France	picot@vegenov.com
PIETERSE, Corné	Utrecht University	Netherlands	C.M.J.Pieterse@uu.nl
PINEDA, Ana	Laboratory of Entomology, Wageningen University	The Netherlands	ana.pineda@wur.nl
PLANCHAMP, Chantal	University of Neuchatel	Switzerland	chantal.planchamp@unine.ch
PLANCOT, Barbara	Laboratory of Glyco-MEV, University of Rouen	France	barbara.plancot@univ-rouen.fr
POËSSEL, Jean-Luc	INRA Génétique et Amélioration des Fruits et Légumes, Avignon	France	Jean-Luc.Poessel@avignon.inra.fr
POINSSOT, Benoit	UMR Plante-Microbe-Environnement INRA 1088/ CNRS 5184/ Université de Bourgogne	France	benoit.poinssot@dijon.inra.fr
POZO, Maria José	Soil Microbiology and Symbiotic Systems, Estación Experimental del Zaidín (CSIC), Granada	Spain	mjpozo@eez.csic.es
PROIETTI, Silvia	Dept. Agrobiology and Agrochemistry, Tuscia University	Italy	s.proietti@unitus.it
PSHYBYTKO, Natallia	Institute of Biophysics and Cell Engineering, NAS of Belarus	Belarus	pshybytko@rambler.ru
REGLINSKI, Tony	Plant & Food Research	New Zealand	tony.reglinski@plantandfood.co.nz

Participant	Inst. affiliaton	Country	Email
REYMOND, Philippe	University of Lausanne	Switzerland	Philippe.Reymond@unil.ch
RIKA, Ozawa	Center for Ecological Research, Kyoto University,	Japan	ozawar@ecology.kyoto-u.ac.jp
ROATTI, Benedetta	Fondazione Edmund Mach, IASMA Research and Innovation Centre	Italy	benedetta.roatti@iasma.it
ROBERT, Christelle	FARCE, Institute of Biology, University of Neuchâtel	Switzerland	christelle.robert@unine.ch
ROBERTI, Roberta	Dipartimento di Protezione e Valorizzazione Agroalimentare, Bologna University	Italy	roberta.roberti@unibo.it
ROBSON, Joanne	Newcastle University	United Kingdom	Joanne.Robson@ncl.ac.uk
ROLLAND, Anne	Bayer SAS	France	anne.rolland@bayer.com
SALDAJENO, Mary Grace	The United Graduate School of Agricultural Sciences, Gifu University	Japan	grace.saldajeno@gmail.com
SASEK, Vladimir	Institute of Experimental Botany AS CR, Prague	Czech Republic	sasek@ueb.cas.cz
SCALSCHI, Loredana Maria	Universitat Jaume I	Spain	scalschi@uji.es
SCHAFFRATH, Ulrich	Department of Plant Physiology, RWTH Aachen University	Germany	schaffrath@bio3.rwth-aachen.de
SCHMITT, Annegret	Julius Kühn-Institut, Institute for Biological Control, Darmstadt	Germany	annegret.schmitt@jki.bund.de
SCHUSTER, Rolf	Helmholtz Zentrum München, Institute of Biochemical Plant Pathology	Germany	rolf.schuster@gertec.eu
SEMIN, Alexander	Russian Research Institute of Phytopathology, RAAS, Moscow region	Russia	semina_yulia@yahoo.com
SEMINA, Yulia	Russian Research Institute of Phytopathology, RAAS, Moscow region	Russia	semina_yulia@yahoo.com
SHAH, Jyoti	Universtiy of North Texas, Department of Biological Sciences, Denton TX	USA	shah@unt.edu
SHCHERBAKOVA, Larisa	Russian Research Institute of Phytopathology, RAAS, Moscow region	Russia	larisa@vniif.ru
SOLANO, Roberto	Centro Nacional de Biotecnologia (CNB-CSIC), Universidad Autonóma de Madrid	Spain	rsolano@cnb.csic.es
SOLER, Roxina	Laboratory of Entomology, Wageningen University	The Netherlands	roxina.soler@wur.nl
SPACOVA, Miroslava	Institute of Experimental Botany AS CR, Prague	Czech Republic	mirca.spacova@centrum.cz
STAVROULAKIS, Stylianos	School of Biology, Newcastle University	United Kingdom	stylianos.stavroulakis@ncl.ac.uk

Participant	Inst. affiliaton	Country	Email
STUMPE, Michael	Plant Biology, Dept. Biology, University of Fribourg	Switzerland	michael.stumpe@unifr.ch
SUGIMOTO, Koichi	Center for Ecological Research, Kyoto University	Japan	sugimok@ecology.kyoto-u.ac.jp
SZABÓ, Anita	Corvinus University of Budapest	Hungary	szanita89@gmail.com
SZALONTAI, Balint	Department of Plant Physiology, Faculty of Sciences, University of Pecs	Hungary	balint@gamma.ttk.pte.hu
TAKABAYASHI, Junji	Center for Ecological Research, Kyoto University	Japan	junji@ecology.kyoto-u.ac.jp
TAKEMOTO, Hiroyuki	Center for Ecological Research, Kyoto University	Japan	takemoto@ecology.kyoto-u.ac.jp
THORDAL-CHRISTENSEN, Hans	Dept. Agriculture and Ecology, University of Copenhagen	Denmark	htc@life.ku.dk
THUROW, Corinna	Albrecht-von-Haller-Institute for Plant Sciences, Georg-August-University Goettingen	Germany	cthurow@gwdg.de
TOMCZYK, Anna	Department of Applied Entomology, Warsaw Agricultural University	Poland	anna_tomczyk@sggw.pl
TON, Jurriaan	Rothamsted Research, Harpenden	United Kingdom	jurriaan.ton@bbsrc.ac.uk
TORRES VERY, Rocio	Estacion Experimental del Zaidin, CSIC, Granada	Spain	rocio.torres@eez.csic.es
TRDA, Lucie	UMR Plante-Microbe-Environnement INRA 1088/ CNRS 5184/ Université de Bourgogne	France	lucie.trda@gmail.com
TYTGAT, Tom	Dept Ecogenomics, Radboud University Nijmegen	The Netherlands	t.tytgat@science.ru.nl
UEDA, Hirokazu	Center for Ecological Research, Kyoto University	Japan	no_hi-ueda@nara.kindai.ac.jp
VALENTOVA, Olga	Institute of Chemical Technology Prague	Czech Republic	olga.valentova@vscht.cz
VAN 'T HOF, Pieter	Université de Fribourg	Switzerland	pieter.vanthof@unifr.ch
VAN PELT, Hans	Plant Microbe Interactions, Utrecht University	The Netherlands	h.vanpelt@uu.nl
VAN VERK, Marcel	Utrecht University	The Netherlands	m.c.vanverk@uu.nl
VAN WEES, Saskia	Plant-Microbe Interactions, Utrecht University	The Netherlands	s.vanwees@uu.nl
VECHET, Lubomir	Crop Research Institute, Prague - Ruzyne	Czech Republic	vechet@vurv.cz
VEYRAT, Nathalie	Institut de Biologie, Université de Neuchâtel	Switzerland	nathalie.veyrat@unine.ch

Participant	Inst. affiliaton	Country	Email
VITERBO, Ada	Hebrew University of Jerusalem	Israel	viterbo@agri.huji.ac.il
VLOT, Corina	Helmholtz Zentrum Muenchen, Institute of Biochemical Plant Pathology	Germany	corina.vlot@helmholtz-muenchen.de
VOS, Irene	Plant Microbe Interactions, Utrecht University	The Netherlands	i.a.vos@uu.nl
WALLING, Linda	University of California, Riverside	USA	linda.walling@ucr.edu
WELTMEIER, Fridtjof	PLANTA GmbH	Germany	m.wolter@kws.com
WIDIASTUTI, Ani	United Graduate School of Agricultural Science, Tokyo Univ. of Agriculture and Technology	Japan	aniwidiastuti@ugm.ac.id
ZAMIOUDIS, Christos	Plant-Microbe Interactions, Utrecht University	The Netherlands	C.Zamioudis@uu.nl
ZANDER, Axel	Université de Fribourg	Switzerland	axel.zander@unifr.ch
ZEIER, Jürgen	Heinrich Heine University Düsseldorf; Department of Biology	Germany	Juergen.Zeier@uni-duesseldorf.de
ZHANG, Wen Jing	Dept. Agriculture and Ecology, University of Copenhagen	Denmark	wzg@life.ku.dk
ZIMMERLI, Laurent	National Taiwan University, Taipei	Taiwan	lauzim2@ntu.edu.tw