1ST JOINT BOTRYTIS-SCLEROTINIA SYMPOSIUM

ABSTRACTS

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OPENING LECTURE

Managing the plant pathogens *Botrytis* and *Sclerotinia* over decades: From the lab to the field and back

Yigal Elad

Department of Plant Pathology and Weed Research, The Volcani Center, Rishon Letzion, Israel

Botrytis and *Sclerotinia* spp. cause various types of diseases (e.g. gray and white molds) on many plant species, on root crown, stem base, stem, leaf, flower organs and fruits. In early modern research, the two genera were systematically-assigned together. They are high humidity-promoted and require a thin film of water for infection by spores. Research and practice of diseases management relies on cultural, chemical, and biological methods, plant nutrition and integration of control means. Aeration, ventilation, air circulation and heating restrict the wetness that is essential for spore germination and infection. Plant spacing, soil polyethylene mulch, and day-time passive heat in non-heated greenhouses result in *B. cinerea* and *S. sclerotiorum* suppression. Interestingly, detached organs of cultural methods-treated plants have reduced susceptibility to both pathogens. Correlations between microclimate parameters and disease calculated for field conditions' epidemics revealed the importance of not only air RH and temperature to the development of the diseases in plant canopies, but also of negative relations with soil temp. This led to the development of a physical root zone treatment that induces plants resistance. Biocontrol with *Trichoderma harzianum* T39 that was developed as stand-alone or as part of integrated management of gray, white molds and other diseases in vineyards and greenhouse crops, utilizes various modes of action. Systemic induced resistance is the leading mode of activity for the bio-fungicide that primes salicylic acid- and ethylene-related gene expression in tomato plants. The role of plant host response to the pathogens was also demonstrated with other suppressors of the diseases. Biochar (product of plant waste pyrolysis) in soil enhanced rhizosphere microbial biodiversity and beneficial microorganisms and induced systemic resistance against the foliar pathogens. It induced priming of early as well as late-acting defense responses, particularly in the genes *Pti5* (ethylene-related) and *Pi2* (jasmonic acid-related), which are crucial in resistance against *B. cinerea*. Nutritional elements such as K, Ca, Mg and K affect the foliar diseases in several plant species and were found to upregulate induced defense signaling pathways and immunity. In conclusion, decades of research and practice revealed varied means of diseases suppression that either directly affect the pathogens, and/or indirectly induce plant-host immunity to the pathogens.

SESSION I: DIVERSITY, HOST ADAPTATION, DEVELOPMENT

Chairs: Jan van Kan & Muriel Viaud

KEYNOTE LECTURE

Diversity and host specialization in *Botrytis* species

Anne-Sophie Walker

Université Paris-Saclay, INRAE, UR BIOGER, Grignon, France

The genus *Botrytis* is highly diverse, with numerous species differing in terms of their biology, ecology and host range. Yet, although one of the oldest, most well studied, and most economically important fungal taxa, many species in this genus have remained obscured for nearly 300 years because of the difficulty in distinguishing these species on the basis of their morphology, using conventional mycological methods. Advances in molecular genetics, and the development of genomics and relevant phylogenetic markers has resulted in the establishment of 38 formally described species and 10 putative species to date. Host specialization is crucial for pathogens. The genus *Botrytis* is remarkable and constitutes a model for plant pathology in that it allows exploration of the determinants of pathogenecity in phylogenetically related species, exploring the full range of host specialization. Indeed, some species are specialists in one plant species or genus, while others, infect a large variety of wild or domesticated plant species, covering multiple botanical families. The fine description of host ranges and the degree of generalism among *Botrytis* species is directly related to the efforts undertaken by our community to explore different ecological niches and to experimentally confirm the pathogenicity of isolates to a wide range of plant species. An initiative is proposed to better compile and share information on the description of host spectra within the genus.

With more than 1400 possible plant hosts recorded, *B. cinerea* is considered a broad generalist. However, recent data have revealed population structure correlated to the host of origin of isolates. This observation raises the hypothesis of ongoing host specialization in a generalist species and may deepen our theoretical knowledge of the evolutionary mechanisms involved in the early stages of population divergence and subsequent speciation. Using genome scans for selective sweeps and divergent selection, tests of positive selection based on polymorphism and divergence at synonymous and non-synonymous sites, and analyses of presence and absence variation, several candidate genes representing possible determinants of specialization to tomato *vs.* grapevine were identified from French isolates. Additionally, accessory chromosomes, repertoires of transposons and their derived small RNAs contribute to the differentiation between populations specialized on different hosts. Finally, GWAS achieved from the interaction between multiple hosts and isolates revealed that *B. cinerea* virulence and host specificity is highly polygenic and involves a diversity of mechanisms.

Population structure of *Sclerotinia subarctica* and *S. sclerotiorum* in England, Scotland and Norway in crop plants and meadow buttercup

John Clarkson[1], Rachel Warmington[1], Peter Walley[2], Matthew Denton-Giles[3], Martin Barbetti[4], Guro Brodal[5] and Berit Nordskog[5]

[1] *Warwick Crop Centre, University Warwick, Warwick, UK.*

[2]*University of Liverpool, Liverpool, UK*

[3]*Centre for Crop Disease Management, Curtin University, Perth, Western Australia.*
[4] *School of Plant Biology, University of Western Australia, Western Australia.*

[5] *Norwegian Institute of Bioeconomy Research, Ås, Norway.*

Sclerotinia species are important fungal pathogens of a wide range of crops and wild host plants. During an initial population study of *S. sclerotiorum* in English crops, isolates were found infecting flowers of meadow buttercup (*Ranuculus ficaria*) and at one site, *S. subarctica* was identified (Clarkson et al., 2010, *Plant Pathology* 59, 1173). Little is known about this related pathogenic species and hence a more extensive study encompassing England, Scotland and Norway was carried out to determine the incidence and population structure of *S. subarctica* in comparison with *S. sclerotiorum* (Clarkson et al., *Frontiers in Microbiology* 8, 490). *S. subarctica* was only identified at one location in England and comprised only 4.3% of *Sclerotinia* isolates in this country, compared to 18.3 and 48.0% in Scotland and Norway respectively. Characterisation using microsatellite markers identified 75 *S. subarctica* haplotypes within a total of 157 isolates over the three countries. Eight of these were shared between Scotland and Norway while none were shared with England. Population analyses suggested a common ancestry of Scottish and Norwegian *S. subarctica* isolates, while English isolates were assigned to a separate population cluster and exhibited low diversity indicative of isolation. Population structure was also examined for *S. sclerotiorum* isolates from England, Scotland, Norway, and Australia. In total, 484 haplotypes were identified within 800 *S. sclerotiorum* isolates with 15 shared between England and Scotland and none shared between any other countries. English and Scottish isolates shared a common ancestry while Norwegian and Australian isolates were assigned to separate population clusters. Sequencing part of the intergenic spacer (IGS) region of the rRNA gene resulted in 26 IGS haplotypes within the 870 *S. sclerotiorum* isolates, nine of which had not been previously identified and two of which were also widely distributed across other countries. *S. subarctica* therefore has a multiclonal population structure similar to *S. sclerotiorum*, but a different ancestry and distribution across England, Scotland, and Norway.

The genetic structure of *Sclerotinia sclerotiorum* in a multihost and year-round agricultural environment.

Rhaphael Alves Silva[1], Miller S. Lehner[1]; Eduardo S. G. Mizubuti[1]

[1]*Universidade Federal de Viçosa, Viçosa, MG, Brazil*

Different host species of *Sclerotinia sclerotiorum* (Ss) are available year-round in tropical countries. Such an environment may lead to a different genetic structure of the population of Ss compared to the structure in temperate regions. The present study was conducted to analyze the population of Ss using individuals from different hosts, grown year-round in a tropical region. More than 200 isolates of Ss from different hosts were genotyped with SSR markers and the mycelial compatibility groups (MCGs) were also characterized. MCGs and multilocus lineages (MLLs) were related, although not completely. There was no structure according to host or geographic region, but the population was found to be structured by MCGs. There is evidence of random mating within MCGs (populations) and the two largests MCGs, MCG 1 and MCG 2, seem to persist in a wide geographic area affecting different hosts. Contrary to what was observed in the current study, the reports from temperate climates do not often report structuring of the population of Ss according to MCG. Apparently, in the tropics, the inoculum sources for white mold epidemics caused by individuals of the two prevalent populations are abundant, presenting a major challenge to disease management and to the breeding programs.

Light-dependent development in *Botrytis cinerea*

Julia Schumacher

Federal Institute for Materials Research and Testing (BAM), Department 4 – Materials and the Environment, Berlin, Germany

Sunlight is an important environmental factor is almost all ecosystems by being a source of energy, information, and stress. All organisms must protect themselves from the harmful effects of light such as UV radiation, ROS accumulation, heat, and desiccation. Finally, light qualities and quantities can be used for decision making, timing and as guide for directed growth when they are sensed and transduced into intracellular signals. *Botrytis cinerea* and other plant pathogens infecting the sun-exposed parts of the plant must cope with the high light conditions the host plant seeks. Further they experience an altered light spectrum ('green gap') when they colonize shaded parts of the plant; it is depleted for blue and red light that is absorbed by the plant chlorophyll and enriched for green and far-red light that is reflected or transmitted by the plant tissue. As these ambient light conditions trigger the shade avoidance response in the plant, the pathogens may trigger their own 'shading response' such as the upregulation of virulence determinants and inoculum production. *B. cinerea* maintains a highly sophisticated light signaling machinery that senses different light qualities to trigger a variety of responses, that are protection, morphogenesis, positive and negative tropisms, and entrainment. These characteristics render *B. cinerea* a valuable model to enlighten the role of light in parasitic fungus-plant interactions and beyond. The vegetative mycelium – the core of all infection and developmental programs – is not visibly pigmented and thus considered to be sensitive to biotic and abiotic stresses. However, the vegetative hyphae have a very limited half-life and are usually restricted to the invasive growth phase in which they are protected from light by the plant tissue. Fast colonization of host tissues and by this proper nutrient acquisition enables the rapid formation of long-lasting reproduction structures (melanized conidiophores with conidia, sclerotia) on the surfaces of rotted plant tissues. Depending on the light and temperature conditions, conidiation or sclerotial development is initiated. Taken together, *B. cinerea* uses light-regulated signaling networks to avoid light whenever possible; for example, by minimizing the half-life of sensitive cells that are hiding in plant tissues and by scheduling critical steps such as conidiogenesis, conidial germination and penetration of plant tissues for the night.

DNA metabarcoding of fungal community associated with *Botrytis*-infected grape berries reveals differences in pathobiome composition between noble rot and grey rot

József Geml[1,2], Ádám I. Hegyi[2], Júlia Hegyi-Kaló[2], Margot Otto[1], Kálmán Z. Váczy[2]

[1]*ELKH-EKKE Lendület Environmental Microbiome Research Group, Eszterházy Károly Catholic University, H-3300 Eger, Hungary*

[2]*Food and Wine Research Institute, Eszterházy Károly Catholic University, H-3300 Eger. Hungary*

Botrytis cinerea is a well-known pathogen of several agricultural crops, causing grey rot in grapevine. However, under certain microclimatic conditions, *Botrytis* infection results in noble rot, an essential process in the production of the sweet and aromatic botrytized wines, such as the Tokaji *aszú* in Hungary. While the traditional view of noble rot and grey rot focuses on the dominant role of *B. cinerea*, former culture-based studies found that several plant pathogenic and saprotrophic fungi also inhabit noble rot grape berries, e.g., *Alternaria*, *Aureobasidium*, *Cladosporium*, *Rhodotorula* species. However, many microbes cannot be cultured using current methods and there are important gaps in our knowledge regarding the composition of the pathobiome of noble rot and grey rot. In this study, we generated and analyzed fungal ITS rDNA sequences with Illumina Novaseq to fully characterize the fungal community associated with healthy, noble rot and grey rot grape berries. Fungal community associated with noble rot and grey rot grape berries strongly differed in diversity and composition from those found in healthy, asymptomatic berries. In addition, even though noble and grey rot berries shared many fungal species, we also found several fungi specific to each rot type. Our results reveal that there is more to noble rot than only *B. cinerea* and other microorganisms may play important roles in the botrytization process.

A conserved cell-cell communication mechanism mediates interspecies interactions in filamentous ascomycete fungi.

Natascha Stomberg, Hamzeh Haj Hammadeh, Antonio Serrano, Ulrike Brandt,

André Fleissner

Institut fuer Genetik, Technische Universitaet Braunschweig, Spielmannstrasse 7, D-38106 Braunschweig.

Cell fusion is essential for the development of most eukaryotic organisms. In axenic culture, germinating spores of *Botrytis cinerea* grow directionally towards each other and fuse to form a supracellular network that develops into the mycelial colony. This type of germling fusion is common in filamentous ascomycete fungi and is thought to increase the competitiveness in natural habitats. A well established model organism to study cell-cell-fusion is *Neurospora crassa.* Fusion germlings of *N. crassa* employ a specific signaling mechanism, which is often referred to as a "cell dialog". In this process, the two fusion partners coordinately alternate between signal sending and signal receiving. This unusual cellular behavior involves the alternating recruitment of the MAP kinase MAK-2 and the SO protein to the plasma membrane.

To test if the "cell dialog" mechanism is conserved in other fungi, we investigated the role of the MAK-2 (BMP1) and SO (BcPro40) homologs in the grey mold *Botrytis cinerea*. In the respective Δ*bmp1* and Δ*bcpro040* gene knockout mutants, germling fusion is absent, comparable to the phenotype of Δ*so* and Δ*mak-2* strains of *N. crassa*. In addition, we observed a similar dynamic membrane recruitment of the two proteins in interacting cell tips, suggesting that the "cell dialog" signaling mechanism is also conserved. When *B. cinerea* and *N. crassa* spores were mixed, interspecies interactions were frequently observed, which resulted in mutual attraction and cell-cell contact, which, however, failed to induce successful fusion. In addition, it was observed that *N. crassa* built up a thickened cell wall at contact point. This surprising observation suggests that the so far unknown signal and receptor mediating the cell-cell communication are also conserved, and that post-contact mechanisms have evolved, which prevent interspecies fusion.

FREQUENCY, a canonical circadian protein with novel supra-circadian roles in the fungus *Botrytis cinerea*.

Aldo Seguel[1], Javier Canales[2], Hanna Müller[1], Rebecca Spanner, Luis F. Larrondo[1].

[1]Millennium Institute for Integrative Biology (iBio), Departamento de Genética Molecular y Microbiología, Facultad de Ciencias Biológicas, Pontificia Universidad Católica de Chile, Santiago, CHILE.

[2] Instituto de Bioquímica y Microbiología, Facultad de Ciencias, Universidad Austral de Chile.

The fungus *Botrytis cinerea* is a relevant necrotrophic phytopathogen from both an economical and scientific perspective. A key element in Botrytis virulence is light, which is able to regulate its development and behavior. Botrytis possesses a circadian clock constituted by the negative regulator frequency (BcFRQ1) and a heterocomplex, that regulates BcFRQ1 expression, composed by White Collar 1 and White Collar 2 (BcWCL1, BcWCL2). When BcFRQ1 is deleted, the *Δbcfrq1* mutant strain turns into an "always sclerotia" phenotype. On the other hand, neither the *Δbwcl1* mutant nor any other FRQ mutants in filamentous fungi have shown this phenotype.

The *Δbcfrq1* phenotype can be reverted by adding primary sources of nitrogen to the media. This evidence suggests that in *B. cinerea* BcFRQ1 serves roles that go beyond its circadian function. In order to determine which metabolic pathways are disturbed in the absence of BcFRQ1, an RNAseq was performed on *Δbcfrq1* strains. Mutant and wild type strains were cultured with or without glutamine, under two different light conditions: constant light (LL), and constant darkness (DD). We observed changes in the expression of 1090 genes when comparing the *Δbcfrq1* strain with the WT (568 genes upregulated, 522 genes downregulated). Most of these genes are related to the mitochondrial function, which has been suggested to be a link between circadian clock and metabolism. These results demonstrate that nutrition assimilation pathways are severely affected in the *Δbcfrq1* mutant. In addition, we have adopted and heterologous approach by expressing *bcfrq1* in a Neurospora *Δ*frq background. Overall, this work can help to understand the evolutionary origins and specialization of FRQ in clock and metabolic crossroads.

Funding: iBIO, FONDECYT 1211715 and HHMI International Research Scholar grant

SESSION I: DIVERSITY, HOST ADAPTATION, DEVELOPMENT

POSTERS

Multiple gene typing analysis identifies distinct genetic lineages within *Botrytis cinerea* population and reveals the epidemiological role of ground cover vegetation in vineyard

Giuliana Maddalena [1], Demetrio Marcianò [2], Alessandro Passera [2], Fabio Quaglino [2], Silvia Laura Toffolatti [2]

[1] *Università degli Studi di Milano, Dipartimento di Bioscienze, via Celoria 26, 20133, Milano, Italy*

[2] *Università degli Studi di Milano, Dipartimento di Scienze Agrarie e Ambientali, via Celoria 2, 20133, Milano, Italy.*

Botrytis cinerea is considered a highly polyphagous pathogen species, even if signatures of host adaptation have been recently described. *B. cinerea* has a complex epidemiology in vineyards, that is influenced by its saprophytic attitude, host phenology, and agronomic practices. Concerning the latter aspect, the influence of ground cover vegetation, including *B*. *cinerea* host plants, on grey mould incidence is controversial. Up to now, no studies on the epidemiological role on grapevine of *B. cinerea* strains coming from alternative hosts is available. The main objective of this study was to investigate the genetic variability of *B. cinerea* strains isolated from non-crop plants and grapevine in order to determine their potential role in the epidemiology of grey mould in vineyard.

Strains of *B. cinerea* have been isolated from symptomatic grapevine samples and spontaneous vegetation present in the inter-rows of a cv Chardonnay vineyard located in Lombardy at Corte Franca (BS). Molecular characterization of the strains has been performed by sequencing portions of several genes: *ITS*, *G3PDH*, BC-*hch, NEP1, NEP2*, and *sdh*B. Mating type, vegetative compatibility, saprophytic attitude, and pathogenicity on grapevine leaves and berries of selected strains were moreover determined.

A total number of 63 strains was isolated from 330 samples collected in field. Of these strains, 50 were isolated from grapevine and only 13 strains from other plant species, mainly belonging to the Brassicaceae family. Phylogenetic analysis showed that the strains isolated from spontaneous vegetation and grapevine, all belonging to the *B. cinerea* species and not differing for saprophytic nor pathogenic attitudes, grouped together. These findings suggest that the strains isolated from spontaneous plants, even if found at low frequency, could play a role in the epidemiology of *B. cinerea* on grapevine.

Intriguingly, based on SNP analysis of BC-*hch* gene sequences, the strains were divided into four lineages. Phylogenetic analysis showed that strains of the lineages 3 and 4 formed a distinct clade inside *B. cinerea* group II. Moreover, strains of such lineages showed different frequencies of *MAT1-1* and *MAT1-2* alleles, and no vegetative compatibility with the other two lineages. It could be hypothesized that *B. cinerea* strains of BC-*hch* lineages 3 and 4 are diverging in a distinct *B. cinerea* subpopulation, but more investigation is needed to confirm this result.

Grape berry texturable changes during the noble rot process

Júlia Hegyi-Kaló[1,2], Ádám István Hegyi[1], József Geml[3], Margot Otto[3], Zsolt Zsófi[1], Xénia Pálfi[1], Kálmán Zoltán Váczy[1]

[1]Food and Wine Research Institute, Eszterházy Károly Catholic University, 6 Leányka Street, H-3300 Eger, Hungary.

[2] Department of Microbiology and Biotechnology, SZIU, 14-16 Somlói street, H-1118 Budapest, Hungary.

[3]MTA-EKE Lendület Environmental Microbiome Research Group, Eszterházy Károly Catholic University, 6 Leányka Street, H-3300 Eger, Hungary.

The well-known grapevine pathogen, *Botrytis cinerea* is causing grey rot of berries, but certain microclimatic conditions, *Botrytis* infection results in noble rot. One of the most renown natural sweet wine, the aszú wines of Tokaj wine region of Hungary is made by the examined noble rot process. The textural characteristics and microorganisms of the grape berry (filamentous fungi and yeasts) associated with berries through several noble rot phases (phase 1-helathy to phase 4-noble rotten) in the two main cultivars grown in Tokaj: *Vitis vinifera* cv. "*Furmint*" and "*Hárslevelű*". We measured the textural parameters routinely tested in viticulture and analysed ITS rDNA sequence data of fungi from the sampled berries. Analysis of responses to different mechanical exposures were made from which, it was cleared out that how the berry skin break force (F_{sk}) and energy (W_{sk}) changes. These texture parameters show decreasing trend from phase 1 to 3 and increasing to phase 4, proofing the practical experiences of the process such as mature, over-mature, botrytising, wither. Berry hardness (BH) play a continuous decreasing role, the elastic modulus (E_{sk}) shows a drop like effect (*E-drop*) between phase 1 and phase 2. Greater diversity in fungal community in case of cv. *Hárslevelű was* found, but the richness of the fungal microbiome become higher over withering in cv. *Furmint*. However, the fungal diversity is decreasing during the noble rotting process the total number colony forming unit of yeast and mould increases. According to the functional gene expression analyses strong effect was found between the *Botrytis cinerea* metabolic function and the berry textural parameters.

This work was founded by the NTP-NFTÖ-21-B-0111.

Heat acclimation in the gray mold fungus *Botrytis cinerea*

Mingzhe Zhang, Amir Sharon

School of Plant Sciences and Food Security, Tel Aviv University, Tel Aviv, Israel

Each species of fungus has a particular range of temperatures in which it can survive. Within this range, there is a narrower temperature range that is considered "optimal" for growth; Sub- or supra-optimal temperatures are considered temperature stress. A shift from optimal to stress temperatures is accompanied by cellular and developmental changes that are necessary for the organism to acclimatize to the new conditions. We investigate mechanisms of heat adaptation in *Botrytis cinerea.* While the optimal growth and infection temperatures of *B. cinerea* are relatively low, the fungus can nevertheless survive periods of much higher temperatures, potentially leading to a reappearance of the disease when temperatures drop to within the optimal range. In a preliminary study we discovered that exposure of the fungus to a moderately high temperature reduces the sensitivity of the fungus to a following, more severe temperature stress. Our goal is to gain molecular understanding of the priming mechanism and learn how helps the fungus to better survive during heat periods. Towards achieving this goal, we characterized fungal response to a range of temperatures and defined temperatures that induce a strong priming effect, namely improve ability of the fungus to cope with potentially lethal temperatures. Using RNAseq and proteomics analyses we were able to identify priming-associated gene and proteins. Among these genes, we identified a subset of serine-type peptidases that were specifically upregulated under priming conditions. Preliminary analysis confirmed that at least some of these peptidases are required for priming. We expect that this study will unravel hidden aspects of temperature-associated *B. cinerea* lifestyles, and lead to a better understanding of mechanisms that regulate fungal heat adaptation. Deciphering the principles and mechanisms that enable *B. cinerea* to survive during periods of heat stress, and to recover and resume growth and infection following such a stress, holds potential for development of temperature-based disease management approaches as alternative to chemical control.

SESSION II: OMICS

Chairs: Jeffrey Rollins & Laurence Godiard

KEYNOTE LECTURE

Comparative genomics of *Sclerotiniaceae*

Jan van Kan

Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands

With the constant improvement of sequencing technologies and supporting bio-informatics tools, there is presently a wealth of genome information for fungi, albeit with different qualities of assembly and annotation. In the fungal community, >1000 genomes have been sequenced and analysed. For some fungi, sequences of hundreds of isolates are available. At present, to my knowledge, there are 21 *Botrytis* species, 3 *Sclerotinia* species and 10 other Sclerotiniaceae for which genomes have been sequenced. The gapless, well annotated genomes of *Botrytis cinerea* and *Sclerotinia sclerotiorum* published in 2017 and 2018 are treasure troves that are exploited by many in our communities. These two genomes serve as references for the other species and help us in designing biological hypotheses that are tested in different groups. I will present a description of the genomes of *Botrytis* species and address the following topics:

- Insights into the host specificity of *Botrytis* species
- The dynamics of secondary metabolite gene clusters within *Botrytis* genomes
- Horizontal transfer of secondary metabolite gene clusters among *Botrytis* species
- A reconstruction of the genome architecture of the common ancestor of the Sclerotiniaceae

I will also illustrate how gene expression analyses boost our insight into biological processes that are crucial in the life cycle of Sclerotiniaceae and I will share my thoughts on the benefits of 'omics' technologies for future research on the biology and ecology of the Sclerotiniaceae.

Botrytis cinerea strains infecting grapevine and tomato display contrasted repertoires of accessory chromosomes, transposons and small RNAs

Adeline Simon[1], Alex Mercier[1], Pierre Gladieux [2], Benoit Poinssot [3], Anne-Sophie Walker[1], Muriel Viaud[1]

[1] *Université Paris-Saclay, INRAE, UR BIOGER, Grignon, France*

[2] *PHIM Plant Health Institute, Université Montpellier, INRAE, CIRAD, Institut Agro, Montpellier, France.*

[3]*Agroécologie, CNRS, INRAE, Institut Agro Dijon, Université Bourgogne Franche-Comté, Dijon, France.*

Botrytis cinerea stands out for having a wide host range and is qualified as generalist. Nevertheless, recent studies suggest that it actually corresponds to co-existing populations that show a certain level of host specialization, as described for the French populations T and G1, specialized on tomato and grapevine, respectively (Mercier *et al.,* 2019, *Env. Microbiol.* 21, 4808–21; Mercier *et al.,* 2021 *Phytopathology,* 111,2355-66).

What are the molecular determinants responsible for such host-specialization? Previous Illumina sequencing data revealed genes under positive selection encoding cellulases, pectinases and enzymes involved in the oxidative stress response suggesting that these activities may contribute to the specialization on tomato. Here, using PacBio sequencing, we produced complete assemblies and annotation of the genomes of strains Sl3 and Vv3 that represent the T and G1 populations in order to identify all possible genomic correlates of host-specialization. Both assemblies revealed 16 core chromosomes that were highly syntenic with those of the reference strain B05.10. The main sources of variation in gene content were the subtelomeric regions and the accessory chromosomes (ACs), especially the AC BCIN19 of Vv3 that was absent in Sl3 and B05.10. The repertoires and density of transposable elements (TEs) were clearly different between the genomes of Sl3 and Vv3 with a larger number of subfamilies (26) and a greater genome coverage in Vv3 (7.7%) than in Sl3 (14 subfamilies, 4.5% coverage). An Helitron-like TE was found in almost all subtelomeric regions of the Vv3 genome, in particular in the flanking regions of a highly duplicated gene encoding a Telomere-Linked Helicase, while both features were absent from the Sl3 and B05.10 genomes. Different retrotransposons in the Sl3 and the Vv3 strains resulted in the synthesis of distinct sets of small RNAs. Finally, extending the study to additional strains indicated that the AC BCIN19 and the small RNAs producing TE Copia_4 and Gypsy_7 are common features of the G1 population that are scarcely if ever found in strains isolated from other populations. This research reveals that ACs, TEs and their derived small RNAs differ between populations of *B. cinerea* specialized on different hosts and paves the way for further studies aiming at investigating the molecular mechanisms underpinning host specialization in a polyphagous pathogen (Simon *et al., BioRxiv*, https://doi.org/10.1101/2022.03.07.483234).

Functional Genomics of *Botrytis* spp.

Matthias Hahn[1]

[1]*Department of Biology, Technische Universität Kaiserslautern, 67663 Kaiserslautern,*

Significant progress has been achieved in recent years in our knowlede of the biology and pathogenic mechanisms of *Botrytis cinerea* and its relatives. This has been possible by the use of advanced technologies that have become available for the research community in recent years. In particular, genome and RNA sequencing and proteomics analyses, as well as improved gene editing and mutagenesis techniques have paved the way for comprehensive investigations of biological processes related to *Botrytis* and its interaction with the host plant and the environment. In my short lecture, I will highlight the development of these key technologies and their applicationd in several fields of *Botrytis* research. At the end, I will outline possible future developments in the use of -Omics technologies that will lead to new levels of understanding of Botrytis biology and improved tools for the protection of plant products from grey mold rot.

Transcriptomic analysis of the *Botrytis cinerea Δbcfet1* strain during *Arabidopsis thaliana* infection provides clues behind hypervirulence.

Jaime Naranjo, Gabriel Pérez, Consuelo Hinostroza, Paulo Canessa

Laboratorio de Genética de Hongos. Centro de Biotecnología Vegetal. Facultad de Ciencias de la Vida. Universidad Andrés Bello, Santiago, Chile.

ANID – Millennium Science Initiative Program - Millennium Institute for Integrative Biology (iBio), Santiago, Chile.

The recently described *Δbcfet1* strain of *Botrytis cinerea* is a hypervirulent mutant that requires plant-derived iron to achieve full infection. BcFET1 (a ferroxidase) and BcFTR1 (an iron permease) form the fungus's Reductive Iron Assimilation (RIA) system. While the *Δbcfet1* strain hypervirulence results in larger necrotic lesions and a more robust oxidative response *in planta*, the molecular determinants that may explain increased virulence are unknown. As plant defense strategies rely on extensive transcriptional reprogramming, and since iron acquisition in both plants and fungi is controlled by transcriptional mechanisms, we choose a dual RNA-seq approach to determine differentially expressed genes (DEG) in the *Δbcfet1* strain during *Arabidopsis thaliana* infection. Surprisingly, after 72 h of infection of *Arabidopsis* plants obtained under iron-sufficiency conditions, the deletion of *bcfet1* led to a very low number of DEGs and very few enriched GO terms, including the "iron transmembrane transport" GO term among upregulated genes. Interestingly, among the top 10 most induced genes, we determined *bcpg5* (endopolygalacturonase), *bclcc2* (laccase), *bcerg13* (second step in mevalonate biosynthesis), and RIA's *bcftr1.* As hypervirulence depends on the plant's iron levels, these results suggest that BcFTR1 may potentially incorporate iron in the absence of *bcfet1* and/or that the hypervirulence of *Δbcfet1* depends on the former protein. Indeed, Fe(III) supplementation of the droplet containing conidia during infection assays increased the lesion caused by *Δbcfet1*, while adding a Fe(II)-specific chelator led to a concentration-dependent reduction. To test if hypervirulence of *Δbcfet1* depends on the permease, we generated the *Δbcfet1+Δbcftr1* double mutant strain. Notably, the double mutant did not reveal substantial differences in terms of on-plate growth rate, displaying wild-type conidiation and a decreased infection capacity and not augmented virulence. We are currently conducting genetic complementation experiments to provide definitive evidence of our observations. In the aggregate, our results indicate that both RIA components are more independent than their orthologs in other fungal systems and that the hypervirulence phenotype of *Δbcfet1* requires iron (II) and *bcftr1*.

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Extracellular vesicles of *Botrytis cinerea*

Amélie de Vallée[1], Jean-William Dupuy[2], Christine Moriscot[3], Christine Rascle[1], Bastien Malbert[1], François-Xavier Gillet[1], Cindy Dieryckx[1], Mathias Choquer[1], Nathalie Poussereau[1], Vincent Girard[1] and Christophe Bruel[1]

[1] *Microbiologie, Adaptation, Pathogénie, UMR5240, Université Lyon 1, CNRS, INSA, Bayer SAS, Lyon, France.*

[2] *Plateforme protéome, Centre de Génomique Fonctionnelle, Université de Bordeaux, Bordeaux, France.*

[3] *Integrated Structural Biology Grenoble, Campus EPN / Institut de Biologie Structurale, Grenoble, France.*

Secretion is a central cellular process in the pathogenicity of *B. cinerea*, and its extracellular proteome has been investigated (Choquer *et al.* 2021, *Environ. Microbiol.*, 23, 2293; de Vallée *et al.* 2019, *Front Microbiol*., 10, 2829). Here we explored the existence of non-conventional secretion in *B. cinerea* and focused on the production of extracellular vesicles by this fungus. In correlation with electron microscopy observations of hyphae grown in solid and liquid media, extracellular vesicles of various morphologies and sizes were isolated. Small RNAs were detected in these vesicles and proteomic analysis identified 673 proteins (membrane and soluble) involved in multiple cellular functions, including transport, metabolism, cell wall synthesis, proteostasis, oxidoreduction and traffic. Leaves confrontation assays indicated no cytotoxicity of the vesicles towards plant cells, but confocal microscopy revealed the capacity of the vesicles at targeting onion epidermal cells. In addition, the vesicles targeted *B. cinerea* itself and some other fungi. This study broadens our view on the secretion capacity of *B. cinerea* and its possible role in inter kingdom or cell to cell communication.

Metatranscriptomic analysis of the noble rot process: from textural changes to antimicrobials

Ádám István Hegyi[a], Margot Otto[b], József Geml[a,b], Júlia Hegyi-Kaló[a], József Kun[c,d], Attila Gyenesei[c], Rian Pierneef[e], Kálmán Zoltán Váczy[b]

[a]*Food and Wine Research Institute, Eszterházy Károly Catholic University, H-3300 Eger, Hungary*

[b]*ELKH-EKKE Lendület Environmental Microbiome Research Group, Eszterházy Károly Catholic University, H-3300 Eger, Hungary*

[c]*Genomics and Bioinformatics Core Facility, University of Pécs, H-7601 Pécs, Hungary*

[d]*Department of Pharmacology and Parmacotherapy, University of Pécs Medical School, H-7624 Pécs, Hungary*

[e]*Biotechnology Platform, Agricultural Research Council-Onderstepoort Veterinary Research, Onderstepoort 0110, Pretoria, South Africa*

Botrytis cinerea is one of the key fungi of grape production, which can lead the formation of noble rot under certain environmental conditions. Noble rot results unique metabolic profile, changes the physical texture and chemical composition. The functional genes during the process have been poorly characterized. We generated metatranscriptomic data from *Furmint* grape variety, from three months in the *Tokaj* wine region, Hungary, representing the four phases of noble rot, from the healthy to the fully dried out berry. Weighted gene co-expression network analysis (WGCNA) was made to link textural parameters with *B. cinerea* functional genes. The clustered genes were significantly enriched characterizing the carbohydrate and protein metabolism of the fungi involved in the breakdown of the berry skin structure. In addition, we identified genes expressed throughout the noble rot process belonging to enriched pathways that allow *B. cinerea* to dominate and proliferate during this state, including sulphate metabolizing genes and genes involved in the synthesis of antimicrobials.

Molecular analysis of *B. cinerea* bioactive peptides and its role during infective process

Almudena Escobar Niño[1], Andrea de Mateo Sanchez[1], Francisco Javier Navas López,

Rafael Carrasco Reinado, Francisco Javier Fernández Acero[1]

[1]*Microbiology & Proteomics Laboratory, Institute for Viticulture and Agri-food research (IVAGRO), Marine and Environmental Sciences Faculty, University of Cádiz, Spain.*

Bioactive peptides are sequences of amino acids generated as the result of the degradation of a protein source by one or several proteases from different microorganisms, the result of this hydrolysis produces peptides with an important role as an agonist and antagonist of opioids, antioxidants, anticoagulants, regulators of concentration of cholesterol, blood pressure, or as antifungals and antibiotics. The potential use of *B. cinerea* to produce "bioactive peptides" has been previously reported. However, its presence and role during the infective cycle is still unclear. This work propose the extraction and characterization of bioactive peptides from by *B. cinerea*. In this objective the obtaining biopeptides will be optimized through the hydrolysis of vegetable proteins as well as it's their phytotoxic efficacy, antibiotic or antifungal properties.

For bioactive peptide production, B. cinerea were cultured in the presence of tomato fruits and glucose as control. Filtrated liquid fractions were loaded in centrifugal devices filters (molecular cut-off >3kDa), peptide mixtures were recovered from the bottom of the device. This peptide mixture was cleaned by acetone precipitation. The peptide mixture was lyophilized and stored at -80 °C.

Obtained bioactive peptides were used in tomato phytotoxic assays. Those experiments shows that *B. cinerea* is able to produce bioactive peptides with phytotoxic and antibiotic activities, either transforming plant proteins (TCW) or "de novo" synthesis (GLU). During the project, extracts that present a high phytotoxic (or antibiotic) activity was separated, analyzed and sequenced in HPLC equipment coupled to LC-MS/MS instrument. This approach will represent, for the first time, the role of these peptides during the infection cycle; and highlight the possible biotechnological application of this process in biomass valorization through the transformation of industrial plant residues in to bioactive compounds.

SESSION II: OMICS

POSTERS

Analysis of *B. cinerea* external vesicles as a potential tool for the infective process

Almudena Escobar Niño[1], Maria Antonia Mateo Marin[1], Rafael Carrasco Reinado,

Francisco Javier Fernández Acero[1]

[1]Microbiology & Proteomics Laboratory, Institute for Viticulture and Agri-food research (IVAGRO), Marine and Environmental Sciences Faculty, University of Cádiz, Spain.

External vesicles (EVs) are a heterogenous class of cytosol-containing membrane secreted particles that supply selection, storage, and protection against degradation. It have been categorised according to their size (i.e. nanovesicles, microvesicles) and their biogenesis (i.e. membrane vesicles; or exosomes). EVs represent a system for the coordinated secretion of a variety of molecules, including proteins with a role in intercellular communication. Their functions has been well characterized in human pathogenic microorganism, while there is only a little knowledge of EVs role in plant-infecting microorganism. In *Fusarium oxysporum* EVs seems to play a crucial role in signalling pathways. In *Botrytis cinerea* has been reported that *A. thaliana* sends small RNAs in extracellular vesicles to silence *B. cinerea* virulence genes. However, nothing has been reported about *B. cinerea* EVs. The objective of the present work is to elucidate the *B. cinerea* EVs production and its proteome description. Firstly, an isolation protocol was optimized by the sequential use of differential ultracentrifugation at 100,000g, filtration (0.45 µm) and ultrafiltration (100 kDa). The optimized protocol was used in the analysis of *B. cinerea* EVs obtained under different growing conditions, glucose (GLU) as a constitutive stage and deproteinized tomato cell wall as a virulence stage. Isolated EVs were visualized by Transmission electron microscopy (TEM) using negative staining. Images from TEM showed morphological differences between EVs from both conditions. EVs fraction were further used for phytotoxicity bioassays and proteins identification by LC-MS. This work represents the first description of *B. cinerea* EVs production and its protein content. Therefore, the analysis of this new proteome will help to unmask new proteins implicated in the infective process of this phytopathogenic fungus.

Use of CRISPR/Cas9 editing to generate mutations in *erg27* gene of *Botrytis cinerea* associated with resistance to hydroxyanilides

Anastasios Samaras[1], Janina Werner[2], Ioannis Rodovitis[1], Matthias Hahn[2], and George Karaoglanidis[1]

[1]Plant Pathology Laboratory, Department of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece. [2]Plant Pathology Group, Department of Biology, University of Kaiserslautern, Kaiserslautern, Germany

Hydroxyanilide fungicides (HAs) constitute one of the major fungicide groups used against *Botrytis cinerea*. However, they are of high risk for resistance development. Several previous studies have shown that the main mechanism of resistance to HAs is target site alteration due to mutations in the *erg27* gene (i.e. F412S, F412V, F412I, T63I), associated with different resistance levels to HAs. Often more than one mutation occurs in the target gene of the same strain with unknown effects on the resistance levels or the fitness of the resistant strains. This study was initiated aiming to determine resistance frequencies to HAs in heavily treated crops in Greece, identify *erg27* mutations in the resistant strains and characterize them after transforming the strains using the CRIPR/Cas9 editing system.

Extensive monitoring in fungal populations from tomato (n= 400), strawberry (n= 320) and pome or stone rootstock seedlings (n= 200) revealed resistance to HAs in frequencies of 4, 35 and 50%, respectively. Sequencing uncovered 18 different mutations in *erg27* of resistant strains. Among them, F412S was predominant, while, interestingly there were isolates possessing double or triple combinations of these mutations.

To characterize these mutations in terms of their effects on sensitivity to fenhexamid and fenpyrazamine, CRISPR/Cas9 genome editing with ribonucleoprotein complexes was used. The editing was performed using a sgRNA in close proximity to the mutation site, a telomere plasmid with hygromycin resistance and a 160 bp repair template with the desired editing. These components were added to protoplasts in a PEG-mediated transformation approach. Hygromycin resistant transformants were isolated and screened for the mutation(s) in *erg27* by digestion and sequencing. After several transfers on hygromycin, followed by stop of selection and loss of the unstable telomere plasmid, homokaryotic strains were obtained possessing the F412S, F412V, F412I, F412C, P250F, E263D, ΔP298 mutations and combinations in the genotype P250F/E263D/F412S. Sensitivity levels of these transformed strains to fenhexamid was measured and compared to that of field strains. Results highlight the accuracy and high efficiency of the CRISPR/Cas9 technology in *B. cinerea.* The use of telomere vectors enables unlimited rounds of editing without the need for selection markers and generation of multiple editing in one gene. With this tool fungicide resistance research can be massively improved.

SESSION III: ECOLOGY AND EPIDEMIOLOGY

Chairs: Philip Elmer & Marc Fermaud

KEYNOTE LECTURE

Botrytis epidemics & disease management: yield losses and action thresholds

Odile Carisse

Agriculture and Agri-Food Canada, Research Development Centre, 430 Gouin Blvd., Saint-Jean-sur-Richelieu, Québec, Canada, J3B 3E6

Despite the economic importance of Botrytis-induced diseases, there are only few reports on yield losses and action thresholds. In the context of increasing fungicide resistance, public pressure to reduce fungicide uses, commercial constraints, and limited low risk fungicides available, informed disease management decisions are more important than ever. The philosophy of IPM consists in using different methods to prevent disease and applying a fungicide only when needed. In other words, a fungicides should be apply only if the cost of not applying it overcomes the expected yield losses. In absence of knowledge on the dynamic of yield losses; how is it possible to apply IPM principle or to make informed decision to manage Botrytis-induced diseased? The reliability of action thresholds has been proven particularly for diseases like onion leaf blight (*Botrytis squamosa*) which have many very short infection-sporulation cycles. The objective of the study was to investigate the relationship between incidence of latent petal (PI) and flower (FI) infections, airborne inoculum concentration (ACC), weather variables, and subsequent yield losses caused by *Botrytis cinerea* in June bearing strawberries. A total of 14 data sets were used to develop and validate models and to establish action thresholds. First, seasonal yield losses were statistically described, and then a lagged regression model was developed to describe the dynamic of yield losses over the harvest period.

There was a significant positive linear relationship between mean airborne inoculum, the mean incidence of latent petal and flower infection and mean yield losses ($R^2 > 0.90$). Among all the variables, the one that had the most influence yield losses were: FI, PI, ACC, duration of period with RH>90%, duration of flower wetness, and duration of period at temperature between 15 and 25C. Depending on variables, best time lags varied between 10 and 30 days. It was possible to build several polynomial distributed lag regression models using combinations of variables. However, models build with only weather variables were less reliable in predicting yield losses. Preliminary field validation of action threshold based on ACC and duration of flower wetness at 15-25C suggested that action thresholds can be used to improve strawberry grey mold management with fewer fungicide applications. Practical applications and need for validation under different environments is discussed.

Methods for the estimation of *Botrytis* grey mould from infected wine grapes.

Christopher C Steel, Yu Qiu, John W. Blackman and Leigh M Schmidtke

Gulbali Institute, School of Agricultural, Environmental and Veterinary Sciences, Charles Sturt University, Wagga Wagga NSW 2678, Australia

Botrytis cinerea (grey mould) results in economic losses to many horticultural crops including grapevines. Quantifying the amount of grey mould that can be tolerated in a batch of grapes before wine quality is compromised is difficult. While visual assessments provide an indicator of grey mould contamination, they are not without their limitations, especially as grey mould often occurs within the interior of the bunch in varieties with tightly packed clusters. This study compared methods for estimating *B. cinerea* in grape tissues. *Vitis vinifera* bunches and detached grapes were inoculated in the laboratory with a *B. cinerea* spore suspension. Following incubation, inoculated and un-inoculated bunches were combined to obtain varying levels of grey mould contamination. Bunches were homogenised and ergosterol quantified by HPLC analysis to determine the amount of fungal biomass present per kg fresh wt. of grapes (Steel, *et al.* 2020, *Australian J. Grape and Wine Res.*, 26, 79-89). Sub-samples of bunch homogenates were then analysed for gluconic acid and qPCR for Botrytis quantification. A lateral flow device (LFD) was used for Botrytis antigen detection. Variability in the qPCR data was evident at infection levels of greater than 6 % on a per berry wt. basis which can potentially be explained by the multinucleate nature of the fungus. Gluconic acid was detected in all un-inoculated grape samples, and it was not always possible to differentiate between healthy grapes and grapes with low levels of grey mould contamination. The amount of Botrytis antigens detected positively correlated with both ergosterol and visual estimations of grey mould with the naked eye. Wine was made from Cabernet Sauvignon grapes with different amounts of grey mould infection and sensory analysis conducted. A loss of wine quality was perceived when wine was made from grapes that had ergosterol levels of > 0.3 mg / kg fresh wt. of grapes, and the signal intensity of the LFD was > 50 using a 1/100 diluted juice sample. Taking less than one hour, detection of grey mould by measuring Botrytis antigens is one of the quickest and genus-specific methods for grey mould estimation.

Viral Cross-species Transmission Results in the Hypovirulence of *Botrytis cinerea*

Yue Deng[1,2], Mingde Wu[1,2], Jing Zhang[1,2], Long Yang[1,2] and Guoqing Li[1,2]

[1] *State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, China.*

[2] *Hubei Key Laboratory of Plant Pathology, Huazhong Agricultural University, Wuhan 430070, China*

Interspecies transmission of viruses is a well-known phenomenon in animals and plants whether via contacts or vectors. In fungi, interspecies transmission between distantly related fungi is often suspected but rarely experimentally documented and may have practical ramifications. *Leptosphaeria biglobosa* and *Botrytis cinerea* are two worldwide plant pathogenic fungi. The former cause black leg on many cruciferous crops, such as oilseed rape (*Brassica napus* L.), whereas the latter has a very wide host range causing gray mold on many economic important crops. In this study, a newly described double-strand RNA (dsRNA) virus (Leptosphaeria biglobosa botybirnavirus 1, LbBV1) found asymptomatic in *L*. *biglobosa* was successfully transmitted to *B*. *cinerea*. LbBV1 has two dsRNA segments, namely dsRNA 1 (6,190 bp) and dsRNA 2 (5,900 bp), possessing spherical virions of about 37 nm in diameter. The cDNA sequences of dsRNA 1 and dsRNA 2 show high sequence identity of 78% and 81% to those of Alternaria botybirnavirus 1 (ABV1) at nucleotide level, respectively, and phylogenetic analysis showed that LbBV1 was also closely related to Botrytis cinerea botybirnavirus 1 (BcBV1), indicating LbBV1 belonged to the genus *Botybirnavirus*. LbBV1 infection in *L*. *biglobosa* was asymptomatic, as no significant differences in colony morphology, radial mycelial growth and pathogenicity were observed between LbBV1-infected and LbBV1-free strains. However, cross-species transmission of LbBV1 from *L*. *biglobosa* to *B*. *cinerea* resulted in the hypovirulence of the recipient *B*. *cinerea* strain t-459-V. The cross-species transmission was succeeded only by inoculation of mixed spores of *L*. *biglobosa* and *B*. *cinerea* on PDA or on stems of oilseed rape with the efficiency of 4.6 % and 18.8 %, respectively. To investigate viral cross species transmission between *L*. *biglobosa* and *B*. *cinerea* in nature, RNA sequencing was carried out on *L*. *biglobosa* and *B*. *cinerea* isolates obtained from *Brassica* samples co-infected by these two pathogens and showed that at least two mycoviruses were detected in both fungal groups. These results indicate that cross species transmission of mycoviruses may occur frequently in nature and result in the phenotypical changes of newly invaded phytopathogenic fungi. This study provides new insights for using asymptomatic mycoviruses as biocontrol agents of their newly invaded fungal pathogens.

White and grey molds, where do you come from?

A comparative study on inoculum of *B. cinerea* and *S. sclerotiorum*.

Christel Leyronas[1], Fabien Halkett[2], Cindy E. Morris[1], Maria Choufany[3] and Samuel Soubeyrand[3]

[1] *INRAE, Pathologie végétale, 84140 Montfavet, France.*

[2] *INRAE-Université de Lorraine, IaM, 54280 Champenoux, France.*

[3] *INRAE, BioSp, 84914 Avignon, France.*

One way to move towards an agriculture that is friendlier to the environment and human health is to optimize the protection of plants against polyphagous diseases. Such progress would involve localizing inoculum sources and the pathway of spores in the atmosphere. However, this task can be challenging, especially if inoculum can originate from different potential sources, such as in grey or white mold caused by *B. cinerea* (*Bc*) and *S. sclerotiorum (Ssc*), respectively.

We collected several hundred isolates of *Bc* and *Ssc* from air and field samples (including both, soil and plant material). We genotyped them and estimated their relatedness to decipher the type of inoculum that causes the symptoms on plants. Moreover, we integrated quantitative data, genetic characteristics, climatic parameters and air mass trajectories to assess if the airborne inoculum had a local or distant origin.

The genetic characteristics did not differ significantly among isolates of *Ssc* collected from air, soil and carrots. Moreover, the genetic differentiation between lettuce and soil isolates of *Bc* decreased over three successive lettuce crops. However, there was no significant correlation between the abundance of *Bc* soilborne inoculum and subsequent disease incidence on the crop. For both crops it seems that the disease was initiated by airborne and by soilborne inoculum.

Viable inoculum of *Bc* and *Ssc*, can be detected from air samples almost throughout the year (96 % and 80% of the sampling days, respectively) even when no susceptible crop was located in the vicinity of the sampling sites. The abundance of this inoculum was significantly correlated with several local climatic parameters. For *Bc,* the variation in abundance and genetic characteristics tended to be linked to the origin of the air masses. For *Ssc*, there was low or no genetic differentiation between isolates collected from four different sites. 700 km could separate collection sites that shared airborne isolates with the same haplotypes. This situation was compatible with the hypothesis of a distant origin of inoculum. Moreover, the results suggest that aerial interconnectivity may be a key to assessing exchanges of isolates between regions.

For both fungi, at the local scale, soilborne and airborne inoculum may be involved in disease development. Sources of airborne inoculum seemed to be local without excluding arrival of spores from distant locations. All these parameters should be considered in order to optimize plant protection.

Development and validation of a mechanistic model of *Botrytis cinerea* on grapevine

Giorgia Fedele[1], Elisa González-Domínguez[2] and Vittorio Rossi[1]

[1] *Department of Sustainable Crop Production, Facoltà di Scienze Agrarie, Alimentari e Ambientali, Università Cattolica del Sacro Cuore, Piacenza, Italy.*

[2] *Horta srl, 29122 Piacenza, Italy.*

Recently, a mechanistic model was developed to predict the severity of Botrytis bunch rot (BBR) epidemics, caused by *Botrytis cinerea*, based on weather conditions, vine growth stages and the different infection pathways of *B. cinerea*. The model, which accounts for conidia production on various inoculum sources and for multiple infection pathways, considers two infection periods. During the first period, from "inflorescences clearly visible" to "berries groat-sized", the model calculates i) the infection severity on inflorescences and young clusters caused by conidia. During the second period, from "majority of berries touching" to "berries ripe for harvest", the model calculates ii) the infection severity of ripening berries by conidia; and iii) severity of berry-to-berry infection caused by mycelium.

The model was validated against 21 epidemics, between 2009 and 2014 in Italy and France, by using a discriminant function analysis (DFA), with 81% of accuracy. The DFA showed that the infection risk estimated by the model during the first infection period strongly influences the BBR severity on mature bunches. The relevance of the infections occurring during the early-stages is strictly related with: i) latent infections of young berries occurring at flowering; ii) the saprophytic colonization by *B. cinerea* of bunch trash; and iii) the spore production by *B. cinerea* on bunch trash.

The model was further validated against 23 independent epidemics in Italy, France, and Spain (1997-2018). Post-harvest incubation assays were also conducted using mature berries without symptoms or signs of BBR, to determine the ability of the model to account for latent infections. The model correctly classified the 65% of epidemics when the classification was based on field assessments of BBR severity. When the model was operated to include BBR severity after incubation assays, its ability to correctly predict BBR severity increased from 65% to >87%, showing that the model correctly accounts for latent infections.

The results overall show that the model is a reliable tool for predicting BBR epidemics; therefore, it may be used for supporting decision-making in scheduling fungicide treatments for controlling *B. cinerea* in vineyards.

Effect of soil water content, soil texture and sclerotia conditioning temperature on myceliogenic germination of Australian *Sclerotinia sclerotium* isolates

Pippa Michael[1], Ashmita Rijal Lamichhane[1], Rachael Crockett[1] and Sarita Bennett[1]

[1] *Centre for Crop and Disease Management (CCDM), Curtin University, Perth, Australia*

Sclerotinia stem rot (SSR) disease, caused by the necrotrophic plant pathogen *Sclerotinia sclerotiorum* Lib. (de Bary), is a major limiting factor in the production of Australian *Brassica napus* L. (canola/rapeseed) and *Lupinus* spp. (lupin) crops, with outbreaks of SSR leading to substantial economic losses through reduction in yield and grain quality. The pathogen can infect plants via two distinct pathways depending on sclerotia (resting body) germination type; either by carpogenic germination to produce ascospores that disperse via air current to invade aboveground tissue, or by myceliogenic germination to produce mycelium that directly invades ground-level (basal) tissues (Derbyshire & Denton-Giles, 2016, *Plant Pathology*, 65, 859-877). As the majority of disease outbreaks are attributed to airborne infection via carpogenic germination of sclerotia, an understanding of the environmental triggers for myceliogenic germination and the impact of basal infection on plants are limited. The objective of this study was to determine the impact of soil texture, soil water content (SWC) and isolate conditioning temperature on the myceliogenic germinability and growth of two *S. sclerotiorum* isolates. The water holding capacity of two soils, sand and clay, was determined as 16 and 29% gravimetric SWC, respectfully. The two isolates used, CU8.20 and CU11.7, were from distinct myceliogenic groupings (MCGs) and dry conditioned at either 50°C for 60 days (Australian summer conditions) or room temperature for 30 days. Isolates conditioned at the two temperatures were placed on the surface of each soil set at either 115, 100 or 85% SWC. Myceliogenic germination was monitored for 21 days, with diameter length measured at day 9. Results indicate that all sclerotia germinated within the time period with isolate, conditioning temperature and their interaction having a significant effect on days to first germination, and soil texture and SWC did not. Isolates conditioned at the cooler temperature produced limited mycelium growth in comparison to those conditioned at 50°C. Whilst soil texture and SWC did not affect germinability or growth of mycelium at tested levels, repeating the experiment using drier SWCs (<85%) would enable the critical moisture level for myceliogenic germination in soil to be determined.

Inoculum potential of *Sclerotinia sclerotiorum* & detection methods

Jonathan S West[1], Gail GM Canning[1], Kevin M King[1] and Caroline Young[2]

[1]*Protecting Crops and Environment Dept., Rothamsted Research, Harpenden AL5 2JQ, UK*

[2]*ADAS Crop Protection, UK*

Dispersal of pathogens to a new host is an essential component of disease epidemics. In the case of *Sclerotina sclerotiorum*, the pathogen survives as sclerotia, which are conditioned by cool moist conditions to germinate in spring to produce apothecia. Spores are released from mature apothecia into the air after a dehumidification event, which is usually caused by a gust of wind blowing into the plant canopy. The seasonal timing of this is difficult but not impossible to predict using weather data. The timing of the onset of spore release can vary by at least one month between the west and the drier east of England. Spore release is greatest in dry weather after wet periods but reduces greatly in prolonged dry periods. Detection of airborne spores provides a more robust form of surveillance and monitoring, which can give farmers a better indication of infection risk. Various sampling and diagnostic methods are increasingly being used to understand this and we are currently testing a field-based system to give same-day data on spore presence based on a LAMP assay. Spores are not only deposited onto petals but are also deposited onto leaves and stems. However, they need a petal to fall onto the spore to provide an undefended food supply, allowing infection of the leaf or stem. Infection takes approximately 4 days for visible disease to appear on leaves or stems from colonised petals.

Session III: Ecology and Epidemiology

POSTERS

Effect of a temporary reduction in nitrogen nutrition on the susceptibility of strawberry to grey mould

Jean-François Bourgeay[1], Marc Bardin[1], François Lecompte[2], Magali Duffaud[1] and Philippe C. Nicot[1].

[1] *INRAE, Pathologie végétale, F-84140 Montfavet, France.*

[2] *INRAE, PSH, F-84914 Avignon, France*

Many studies show that the level of nitrogen fertilization of plants influences their susceptibility to pathogens. On strawberry, it was shown that reduced nitrogen fertilization applied continuously for several weeks significantly reduced the susceptibility of the leaves to *Botrytis cinerea* (Nicot et al. 2013, IOBC-WPRS Bulletin 88:39-42). However, a continuously low nitrogen fertilization regime can hardly be applied in production because it may be detrimental to strawberry yield. A temporary reduction in nitrogen nutrition would be preferable but its effect on the level of plant susceptibility is not known. The objective of this study was therefore to determine the time needed for reduced nitrogen fertilization to have an effect on the susceptibility of strawberry to *B. cinerea*.

To address this objective, strawberry plants (varieties Candiss and Darselect) were subjected to three levels of nitrogen nutrition (0.5mM, 5mM, 10mM), following an initial period with a regular fertilization regime (10mM nitrogen). Plant susceptibility was then assessed weekly, using a detached-leaf assay with two strains of *B. cinerea* differing in their aggressiveness. In two independent trials, we observed a rapid onset of the beneficial effect of nitrogen reduction on plant susceptibility. As early as one week after the application of the low nitrogen regime, a reduction of up to 28% in lesion size was recorded on the leaves, in comparison with leaves from plants maintained under the regular fertilization regime. The effect was enhanced over time, with reductions in lesion size of up to 68% after 4 weeks of low nitrogen nutrition. This effect was different depending on the strain of *B. cinerea* and the strawberry variety. Possible hypotheses and the relevance of this study for the integrated protection of strawberry will be discussed.

Key words: Strawberry, *Botrytis cinerea,* nitrogen fertilization

Development and validation of a mechanistic model accounting for the effect of soil moisture, weather and host growth stage on the development of *Sclerotinia sclerotiorum*.

Irene Salotti[1] and Vittorio Rossi[1]

[1] *Department of Sustainable Crop Production (DIPROVES), Università Cattolica del Sacro Cuore, via Emilia Parmense, 84, 29122, Piacenza, Italy.*

Sclerotinia sclerotiorum (Lib) de Bary is a ubiquitous pathogen that causes disease on > 500 host plants distributed worldwide. The control of these diseases is difficult because of the long-term persistence of sclerotia in the soil, which represent the primary inoculum and produce air-borne ascospores able to infect host plants. Combination of cultural practices and applications of biocontrol agents for reducing the number of sclerotia in soil, and fungicide sprays against ascosporic infections are the main disease management options. Mathematical models provide a better understanding of the environmental conditions that are conducive to production of apothecia and infection by ascospores and may improve the timing of fungicide applications so reducing the unnecessary sprays.

By using the systems analysis, we developed a new mechanistic, dynamic, weather-driven model for the prediction of *S. sclerotiorum* epidemics on different crops by mobilizing the available knowledge retrieved through a systematic literature review. The model accounts for i) the production and survival of apothecia; ii) the production, dispersal, and survival of ascospores; iii) infection by ascospores; and iv) lesion onset. The ability of the model to predict the occurrence of apothecia was evaluated for epidemics observed with different climates, soil types, and host crops (soybean, white bean, and carrot) using independent data obtained from trials conducted in Ontario (Canada) in 1981, 1982, and from 1999 to 2002; in Michigan (USA) in 2015 and 2016; and in Wisconsin (USA) in 2016. The model showed 0.82 accuracy and 0.73 specificity in predicting the presence of apothecia, with a posterior probability of correctly predicting the presence or absence of apothecia of 0.804 and 0.876, respectively; prediction errors were only a few days earlier than or later than the real observations. The model was also validated for its ability to predict disease progress on soybean and sunflower in Ontario (Canada) in 1981 and 1982, in Manitoba (Canada) in 2001 and 2002, and in Michigan (USA) in 2015 and 2016. Comparison of model output with real observations showed a concordance correlation coefficient of 0.948, and a root mean square error of 0.122.

Overall, the model provided an accurate (i.e., it provided predictions close to reality) and robust (i.e., it provided accurate predictions in a range of environments and epidemiological conditions) representation of the real system, and could support decision making for disease control.

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Visualization of the early stages of infection of *Botrytis squamosa*, *Botrytis aclada* and *Sclerotium cepivorum* on onion reveals distinct biology and infection strategies

Maikel B.F. Steentjes[1], Olga E. Scholten[2] and Jan A.L. van Kan[1]

[1] *Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands.*

[2] *Wageningen University, Plant Breeding, Wageningen, The Netherlands.*

Botrytis squamosa, Botrytis aclada and *Sclerotium cepivorum* are three fungal species of the family Sclerotiniaceae that are all host-specific pathogens of onion. Despite their close relatedness, these fungi cause very distinct diseases, respectively called leaf blight, neck rot and white rot, which pose serious threats to onion cultivation. *Botrytis* leaf blight is a foliar disease of onion that is initiated by spores that land on the surface of onion leaves and subsequently germinate and penetrate the surface to colonize host tissue. *Botrytis* neck rot is a postharvest disease that occurs in stored onion bulbs without displaying symptoms during the growing season of onion plants. In total three *Botrytis* species are associated with neck rot; *B. aclada*, *B. allii*, and *B. byssoidea*. White rot is a soil-borne disease of onion caused by *S. cepivorum*. Sclerotia of *S. cepivorum* can stay dormant in the soil for many years and germinate upon induction by root exudates of onion plants growing nearby. Initial infection occurs via the roots of the plant and the fungus grows towards the bulb which eventually becomes covered in white mycelium. The infection biology of particularly neck rot and white rot is poorly understood.

In this study, we created GFP-expressing transformants of all three fungi that allow to trace and visualize the early phases of infection. *B. squamosa* entered onion leaves by growing either through stomata or into anticlinal walls of onion epidermal cells. *B. aclada* did not penetrate the leaf surface but instead formed superficial colonies which produced new conidia. *S. cepivorum* entered onion roots via infection cushions and appressorium-like structures. In the non-host tomato, *S. cepivorum* also produced appressorium-like structures and infection cushions, but upon prolonged contact with the non-host the infection structures died. With this study, we have gained understanding in the infection biology and strategy of each of these onion pathogens. Moreover, by comparing the infection mechanisms we were able to increase insight into how these closely related fungi can cause such different diseases.

SESSION IV: HOST-PATHOGEN INTERACTION – VIRULENCE FACTORS

Chairs: Barbara Blanco-Ulate & Nathalie Poussereau

KEYNOTE LECTURE

Mechanisms of *Sclerotinia* virulence and compatibility

Jeffrey A. Rollins

University of Florida, Plant Pathology Department, Gainesville, FL, USA

Sclerotinia sclerotiorum has long been viewed as a model of necrotrophic pathogenesis. Much of our understanding of this necrotrophy centers on the dynamic production and secretion of oxalic acid. A myriad of activities have been associated with oxalic acid during pathogenesis. These activities range from calcium ion chelation to induction of host programmed cell death and inhibition of host autophagy. Gene-specific deletion mutants have indeed demonstrated that oxalic acid is required for full macerating colonization of host tissue yet in some hosts oxalic acid minus mutants produce spreading lesions while restricted lesions are observed in other hosts. These studies demonstrate that oxalic acid is required for macerating colonization, but is not required for establishing infection and basic compatibility with host plants. From these, we are challenged with determining what other factors contribute to virulence and determining whether they function independent of oxalic acid. The identities of a number of virulence gene products and, in a few cases their cellular targets, have been elucidated by several research groups. Despite this significant progress, real challenges remain in understanding when and where these factors function during disease development. A better understanding of the infection and colonization process of *S. sclerotiorum* is needed along with insights into a number of outstanding questions including whether a true "biotrophic" phase of infection exists; what parameters govern ascospore infection of floral tissues; to what extent are non-host species asymptomatically colonized; does colonization of the vascular system allow escape from host recognition; what factors underlie host specificity. Through the pursuit of answers to these and other questions, a more refined understanding of *Sclerotinia* infection and colonization is emerging and will continue to emerge. This knowledge will better pinpoint the forms and timing of host resistance that may lead to effective and durable resistance.

A MADS-box transcription factor gene expressed in infection cushions impairs *Sclerotinia sclerotiorum* virulence

Laurence Godiard[1], Maurinne Payet[1], Malick Mbengue[2], Shantala Mounichetty[1], Adelin Barbacci[1] and Sylvain Raffaele[1]

[1]*Laboratoire des Interactions Plantes Microbes Environnement LIPME, INRAE–CNRS, Castanet-Tolosan, France*

[2]*Laboratoire de Recherche en Sciences Végétales, Castanet-Tolosan, France.*

Sclerotinia sclerotiorum is a generalist plant pathogen, responsible for white mold diseases on many cultivated species. An improved understanding of the molecular basis of *S. sclerotiorum* virulence will lead to conceptual advances in plant pathology and renewed opportunities for engineering durable disease resistance in crops. *S. sclerotiorum* also infects the model plant *Arabidopsis thaliana*, which we use as a plant model to decipher *S. sclerotiorum* virulence molecular components at early infection stages. *S. sclerotiorum* differentiate specific multicellular appressoria, referred to as infection cushions (ICs) that are dedicated to the penetration of host tissues. In order to identify key determinants of IC formation in *S. sclerotiorum*, we exploited RNAseq in multiple conditions to find 55 *S. sclerotiorum* genes expressed specifically in ICs. To document the taxonomic conservation of *S. sclerotiorum* IC-specific genes, we used Blastp searches to look for conservation among 602 fungal proteomes. Among the ten IC-specific genes with reduced taxonomic distribution, we selected a MADS-box putative transcription factor called *SsMADS4* strongly induced in ICs. *S. sclerotiorum* mutants deleted of or overexpressing *SsMADS4* have been generated. Functional analysis of this putative TF supports a role in IC number regulation and host virulence. Further functional studies of *SsMADS4* mutants should help to get insight in the interweaving between fungal virulence and IC formation in *S. sclerotiorum* and other Leotiomycetes.

What is the role of *Botrytis cinerea* Argonautes in host plant infection?

An-Po Cheng[1], Lihong Huang[1] and Arne Weiberg[1]

[1] *Genetics, Faculty of Biology, Ludwig Maximilians University Munich, Martinsried, Germany*

Argonaute (AGO) proteins mediate post-transcriptional gene regulation via small RNAs (sRNA) by sequence homology, known as RNA interference (RNAi). *Botrytis cinerea* is a model organism to study cross-kingdom RNA interference (ckRNAi) in fungal-plant interactions. During plant colonization, *B. cinerea* delivers small RNAs (*Bc*sRNAs) into host plant cells to suppress plant immunity-related genes for infection. To target plant genes, *Bc*sRNAs hijack the plant's own Argonaute (AGO)/RNA-induced gene silencing (RISC) machinery. In a counter-defense mechanism, plants also send sRNAs into *B. cinerea* to suppress infection, making the ckRNAi concept bi-directional. We identified four family members of *Bc*AGOs in *B. cinerea*. What is the role of these *Bc*AGOs during plant infection as well as bidirectional ckRNAi is currently unknown.

In this project, we are phenotyping *bcago* knockout (ko) mutants during plant infection and have recorded mRNA as well as sRNA transcriptome analyses to understand the role of *Bc*AGOs during plant interaction. These analyses provide us first insights, how post-transcriptional gene regulatory *Bc*AGO complexes might control host infection. Moreover, we are profiling protein interaction partners of *Bc*AGOs to unravel post-translational regulation processes of *Bc*AGOs.

Sclerotinia sclerotiorum inoculation response on *Brassica napus* varieties is influenced by environmental conditions

Sarita Bennett[1], Ashmita Rijal Lamichhane[1], Rachael Crockett[1] and Pippa Michael[1]

[1] *Centre for Crop and Disease Management (CCDM), School of Molecular and Life Sciences, Curtin University, Perth, Australia*

Brassica napus L. (canola/rapeseed) is an important break crop in temperate Australia, with annual production of about 3 Mt. Western Australia (WA) is the main canola growing state, producing about 40% of the annual total. *Sclerotinia sclerotiorum* Lib. (de Bary), a widespread necrotrophic fungus with a host range of over 400 species (Bolton et al. 2006, Molecular Plant Pathology, 7, 1-16), causes Sclerotinia stem rot (SSR) disease in canola, which in severe infection years can result in significant yield loss. Aggressiveness of isolates of *S. sclerotiorum* and resistance of canola varieties are typically assessed by determining growth of lesions on the main stem following inoculation using actively growing mycelium (Denton-Giles et al. 2018, Can. J. Plant Path. 40, 1-11). However, previous work by Michael et al. (2022, Phytopathology, submitted) suggests that results are not consistent under different environmental conditions.

The aim of the study was to determine the impact of time of sowing of canola on isolate aggressiveness. Six Australian canola varieties were sown at three different times of sowing (TOS). Varieties were inoculated with four WA *S. sclerotiorum* isolates, plus a control, at 30% flowering. Lesion length was recorded weekly for 28 days and used to calculate Area Under the Disease Progression Stairs (AUDPS). Plants were harvested at the end of the growing season with canola seed and sclerotia production variables recorded.

ANOVA of AUDPS showed there were significant differences ($P<0.05$) between *S. sclerotiorum* isolates and sowing dates, with significant interactions between sowing date and isolate, and between sowing date and variety. AUDPS was greatest at TOS2 and TOS3, with variation between replicates low at TOS2. AUDPS of isolate CU11.19 was low in TOS1 and 3, but significantly greater at TOS2. Seed production was significantly ($P<0.005$) greater at TOS1 and lowest at TOS3. However, in TOS2 all inoculated plants had significantly lower yields compared to the control. At TOS3 there were no differences in yield between inoculated and control plants. However, the most sclerotia within the stem were recorded in TOS3 in all four isolates.

In conclusion, time of inoculation does affect the aggressiveness of isolates and sclerotia production, with CU11.19 in particular showing differences in aggressiveness of AUDPS in relation to environmental conditions following inoculation. AUDPS does not clearly relate to final yield, particularly at TOS3.

The early secretory pathway associated proteins SsEmp24 and SsErv25 regulate morphogenesis and pathogenesis in *Sclerotinia sclerotiorum*

Chong Xie[1,2], Yannong Xiao[2], Gaofeng Wang[2], Jiatao Xie[1,2], Daohong Jiang[1,2] and Xueqiong Xiao[1,2]

[1]*State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Wuhan 430070, Hubei Province, China*

[2]*Hubei Key Laboratory of Plant Pathology, College of Plant Science and Technology, Huazhong Agricultural University, Wuhan 430070, Hubei Province, China*

Sclerotinia sclerotiorum is a destructive necrotrophic phytopathogenic fungus with worldwide distribution and a broad host range of more than 700 plant species. In the previous study, we generated gene *Ss-caf1* disruption mutant Sunf-MT6. The mutant Sunf-MT6 was unable to form infection cushions and disable to infect intact host plants. Recently, we found four genes, *SsErp1*, *SsEmp24*, *SsErp3*, and *SsErv25* that encode for p24 proteins were highly up-regulated at the early stage of infection cushion formation and down-regulated in the infection cushion defective mutant Sunf-MT6. Members of the p24 family are major constituents of COPI- and COPII-coated vesicles, cycle between Endoplasmic reticulum and Golgi in the secretory pathway. Proper secretion of proteins is critical for fungal development and pathogenesis. However, the potential roles of proteins involved in the early secretion pathway are poorly understood in the phytopathogenic fungi. Therefore, the biological function of two p24 proteins, SsEmp24 and SsErv25, in *S. sclerotiorum* was molecularly characterized. SsEmp24 interacts with SsErv25, and both of them predominantly co-localized in the endoplasmic reticulum or nuclear envelope. Both *SsEmp24* and *SsEmp25* mutants displayed abnormal colonies on the artificial medium. *SsEmp24* mutants had more severe vegetative growth and sclerotial formation defects than *SsEmp25* mutants. The *SsEmp24* and *SsEmp25* mutants were defective in infection cushions, which resulted in lower virulence on the healthy or wound leaves of rapeseed and soybean. All phenotypic deficiencies could be restored when the complete coding sequences of *SsEmp24* and *SsErv25* were introduced into the knock-out mutants, which suggested that *SsEmp24* and *SsEmp25* play essential roles in the development, infection cushion, and pathogenesis of *S. sclerotiorum*. Taken together, our research indicated that the proteins related to the early secretory pathway as potential targets to control fungal diseases effectively.

A new secretory pathway in *Botrytis cinerea*: importance of the AP-1/Clathrin machinery in the virulence of the pathogen

Glen Calvar[1], Adrien Hamandjian[1], Eytham Souibgui[1], Mélanie Crumière[1], Christine Rascle[1], Amélie de Vallée[1], Mathias Choquer[1], Christophe Bruel[1] and Nathalie Poussereau[1]

[1]Univ Lyon, Université Claude Bernard Lyon 1, INSA-Lyon, CNRS, UMR5240 MAP, F-69622, France

The secretory pathway plays an important role in hyphal growth and virulence of filamentous fungi through the delivery of cell wall synthesis enzymes and virulence factors both transported in vesicles. In metazoa and yeast, secretory vesicles formation occurs at the Golgi apparatus and endosomes through a complex and partially characterized machinery involving clathrin and adaptor proteins such as AP-1. This adaptor protein is known to recruit clathrin and to sort cargo proteins in many organisms. In filamentous fungi, data on the molecular mechanisms involved in the secretion process are still scarce. Using a mutant underexpressing the clathrin heavy chain encoding gene, we have previously demonstrated the essential role of clathrin in the secretion of virulence factors in *Botrytis cinerea* (Souibgui *et al.* 2021, *Frontiers in Plant Science*, 12:668937). Besides, clathrin seems to be involved in cell wall integrity maintenance as this mutant shows a cell wall defect. Altogether, these results suggest that clathrin may play a role in forming secretory vesicles associated with hyphal growth and pathogenicity. However, these observations made in the clathrin mutant need to be further addressed to ensure that they are related to an abnormality in secretory vesicles biogenesis. A conditional mutant of the β-subunit of the heterotetrameric AP-1 clathrin adaptor complex was therefore constructed. Characterization of the AP-1 mutant revealed a strong alteration in radial growth, hyphal morphology, and a chitin defect associated with a mis-localization of the chitin synthase BcCHSIIIa. Besides cell wall defects, the AP-1 mutant is strongly affected in pathogenicity, infection cushions formation, and secretion. This study demonstrates the essential role of the clathrin adaptor AP-1 in both cell wall integrity maintenance and the infectious process of the plant-pathogenic fungus *B. cinerea*.

Souibgui E, Bruel C, Choquer M, de Vallée A, Dieryckx C, Dupuy JW, Latorse MP, Rascle C, Poussereau N. Clathrin is important for virulence factors delivery in the necrotrophic fungus *Botrytis cinerea*. *Front. Plant Sci* (2021). 12:668937

Small secretory proteins in the pathogenesis of *Sclerotinia sclerotiorum*

Jiasen Cheng[1,2], Yanping Fu[2], Jiatao Xie[1,2] and Daohong Jiang[1,2]

[1] *State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Hubei Province, Wuhan, China*

[2] *Hubei Key Laboratory of Plant Pathology, Huazhong Agricultural University, Hubei Province, Wuhan, China.*

Sclerotinia sclerotiorum is a typical necrotrophic fungal pathogen and has a wide host range including rape and soybean. Previous studies have shown that the cell wall degrading enzymes and oxalic acid play major roles in the pathogenesis of *S. sclerotiorum*. Recently, we found that small secretory proteins, such as SsSSVP1, SsCP1 and SsITL, also are important virulence factors of *S. sclerotiorum*. Knockout or silencing of these protein encoded genes reduced the virulence of *S. sclerotiorum.* SsSSVP1 and SsCP1 could induce significant plant cell death. SsITL could suppress host immunity at the early stages of infection. We clarified the roles and mechanism of these proteins by screening host targets, protein localization, gene expression and plant resistance analysis. SsSSVP1 interacts with a plant mitochondrial protein QCR8 and hijacks it into cytoplasm, thereby disable its biological functions and induce cell death (Lyu et al. 2016, PLoS Pathogens 2(2): e1005435). SsCP1 interacts with PR1 in the apoplast and then maybe inhibiting the potential antifungal activity of PR1 (Yang et al. 2018, New Phytologist 217: 739-755). SsITL interacts with a chloroplast-localized calcium-sensing receptor (CAS) in chloroplasts, and thereby reduces salicylic acid accumulation during the early stage of infection (Tang et al. 2020, Molecular Plant Pathology 21(5): 686-701). These secretory proteins tend to attack the conserved proteins widely existing in host plants, which is consistent with the wide host range of *S. sclerotiorum*. Interestingly, we found that a protein secreted by *S. sclerotiorum* interacted with the cysteine-rich receptor-like kinase 2 (AtCRK2), and it was named CRKIP (CRK2 Interacted Protein). *SsCRKIP* was dramatically up-regulated during the early stage of infection. The knockout mutant Δ*SsCRKIP* showed reduced virulence, However, the virulence of mutant Δ*SsCRKIP* in *SsCRKIP*–expressing transgenic plants is not significantly different from that of wild type strain 1980. Further results showed that SsCRKIP inhibits chitin-induced defence responses including ROS induction, mitogen-activated protein kinase phosphorylation and AtCRK2-mediated H_2O_2 accumulation. Together, our findings could also help us to understand the pathogenic mechanism of necrotrophic fungal plant pathogen from a new perspective.

Novel regulatory genes in *Botrytis cinerea* identified through the characterization of natural non-pathogenic, light insensitive, field isolates

Virginia Casado del Castillo[1], Wilson Acosta Morel[1], Sioly Becerra[1], Jan van Kan[2], José María Díaz Mínguez[1] and Ernesto Pérez Benito[1]

[1]*Department of Microbiology and Genetics, Institute for Agribiotechnology Research (CIALE), Universidad de Salamanca, Salamanca, Spain.*

[2]*Laboratory of Phytopathology, Wageningen University, Wageningen, The Netherlands.*

The natural populations of *Botrytis cinerea* are highly diverse. A number of field isolates altered in pathogenicity were identified when monitoring the incidence of gray mold in the vineyards in Castilla y León (Spain). Genetic tools are being applied on their characterization.

Complementation analysis carried out between eight isolates non-pathogenic on *Vitis vinifera* grouped them in two complementation groups. The first one includes seven mycelial isolates, which do not sporulate, do not produce sclerotia and do not infect any host plant. The second one is defined by a single isolate with a characteristic hyperconidiating phenotype, which does not produce sclerotia, does not infect *V. vinifera* leaves and shows reduced aggressiveness on *Phaseolus vulgaris*. All these mutants are insensitive to light. Genetic analysis in crosses between a representative isolate of each group and an aggressive field isolate, informed that in each case the phenotypes observed are under the control of a single genetic locus. The gene altered in the mycelial non-pathogenic isolates was identified by applying a bulked segregant analysis based strategy and found to be Bcin04g03490, which encodes a protein with a Gal4 DNA binding domain and a maltose acetyltransferase domain. Sequence analysis revealed the nature of the mutations leading to the loss of function in the mutant alleles. Phylogenetic relationships analysis demonstrates that the gene is specific of the Pezyzomycotina within the Ascomycetes. RNAseq analysis informs about a wide regulatory role for the encoded protein controlling numerous genes often adjacently located and regularly spaced along the chromosomes. Since a reduction of fitness is expected for such mycelium non-pathogenic isolates, questions arise concerning the processes and mechanisms by means or which they arise and how they are maintained in the field. The phenotype of the isolate belonging to the second complementation group resembles that of the mutants deficient in the *B. cinerea* white collar 1 gene but functional complementation analysis indicates it is altered in a different gene. These results support a connection between light perception and regulation of developmental processes and pathogenicity in *B. cinerea* and bring to light the role of two novel regulatory genes controlling these processes.

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Lytic polysaccharide monooxygenases are virulence factors of plant infection by necrotrophic pathogens

Jun Zhou, Federico Sabbadin, Simon McQueen-Mason and Katherine Denby

Centre for Novel Agricultural Products (CNAP), Department of Biology, University of York, York, UK

The generalist necrotrophic fungus *Botrytis cinerea* is responsible for significant losses in crop yields around the globe. *B. cinerea*, like many other pathogenic fungi, possess a range of cell wall degrading enzymes to attack and penetrate plant hosts. Lytic polysaccharide monooxygenases (LPMOs) are copper-dependent enzymes able to oxidatively cleave crystalline cell wall polysaccharides such as cellulose and chitin, allowing their subsequent degradation by hydrolytic enzymes. Multiple classes of LPMOs have been identified and recently a family (AA17) of LPMOs was shown to degrade pectin and play a crucial role in infection of potato by the oomycete *Phytophthora infestans* (Sabbadin *et al*. 2021 *Science*, 373, 774-779). The biological role of LPMOs in necrotrophic pathogens, and the use of these genes to control infection is largely unknown. Comparative genomic analysis showed that LPMO families have expanded in necrotrophic plant pathogens compared to fungi with different lifestyles. Here, we have used transcriptome data to identify target LPMOs in *B. cinerea*, and RNAi to silence the expression of these genes during early pathogen infection. Six LPMO-encoding genes of *B. cinerea* (BcAA11_1, BcAA11_2, BcAA16, BcAA9_5, BcAA9_6, BcAA9_7) were up-regulated early during infection of lettuce and co-expressed in diverse lettuce lines with varying resistance and susceptibility. These genes also show a positive correlation of expression with lesion size during infection of Arabidopsis by 96 isolates of *B. cinerea*. We demonstrated that dsRNA against these LPMO genes significantly decreased fungal infection and disease symptoms in lettuce and Arabidopsis. Furthermore, orthologous LPMO genes were also tested in the closely related species, *Sclerotinia sclerotiorum*, and again dsRNA application reduced lesion size indicating likely conserved function across these two fungal pathogens. Collectively, these findings provide evidence for LPMOs playing crucial roles as virulence factors in plant-necrotrophic pathogen interactions, and could help inform future strategies for crop protection and food security.

Selection of Botrytis cinerea *pathogenicity/virulence genes for their knockdown by topical applications of double-stranded RNA*

Maria Spada, Diego Palpacelli, Claudio Pugliesi, Marco Fambrini and Susanna Pecchia

DAFE Department of Agriculture, Food and Environment, University of Pisa, Italy

Botrytis cinerea is a pathogen of wide agronomic and scientific importance also due to its tendency to develop fungicide resistance. Recently, there is great interest in the use of RNA interference as a control strategy against *B. cinerea*. So far, some genes have been targeted for RNAi studies (*e.g.* effectors, cell wall elongation, ergosterol and chitinase biosynthesis, vesicle trafficking pathway genes). In order to reduce possible effects on non-target species, the sequence-dependent nature of RNAi can be used as an advantage to customize the design of dsRNA constructs. In our research, to minimize off-target problems, we selected three pathogenicity/virulence-related *B. cinerea* genes. Firstly, we selected *BcBmp3*, a gene with a key role in pathogenicity, and we demonstrated that our dsRNA targeting this gene was effective in controlling *B. cinerea* and was also highly specific (Spada *et al.* 2021, *Int. J. Mol. Sci.*, *22*, 5362). Moving on, we selected two other genes: *BcPls1* (a tetraspanin related to appressorium penetration) and *BcBmp1* (a MAP kinase essential for fungal pathogenesis), known in the literature for their use in knockout experiments. We performed a predictive analysis of small interfering RNAs for the two dsRNA constructs targeting *BcPls1* and *BcBmp1* that we built up. We synthesized the long dsRNAs *in vitro*, and we applied them locally both to the axenic culture of *B. cinerea* and to artificially inoculated lettuce leaves. *In vitro*, the *BcBmp1* expression level was significantly reduced after dsRNA applications as the mRNA level was 10.4% at 48 hours and 14.8% at 96 hours. Conversely, *in vitro*, a non-significant reduction in *BcPls1* expression was detected at both times. *In vivo*, the expression level of both genes was significantly reduced after dsRNA applications: 11% for *BcBmp1* and 15% for *BcPls1*. Notably, the detached lettuce leaves treated with our dsRNA constructs showed a huge reduction of necrotic areas compared to controls: 86.6% for *BcBmp1* and 87.4% for *BcPls1*. Therefore, we started spraying our dsRNA constructs, naked or loaded onto nanosheets, on lettuce plants and the first results collected for *BcBmp3*-dsRNA are encouraging since a significant reduction of grey mould severity was observed. Other spraying experiments are underway using the *BcBmp1*-dsRNA and *BcPls1*-dsRNA constructs. Considering the need for a high amount of dsRNA for large-scale experiments, we are working on *in vivo* dsRNA production using engineered *E. coli* strains.

Multiple tolerance mechanisms to the plant saponin α-tomatine in *Botrytis cinerea*

Yaohua You[1], Suraj Hassan Muralidhar[1], Andrea Lorena Herrera Valderrama[1], Paul Ruigrok[1], Linda Matz[3], Oostlander Anne[3], Xiaoqian Shi[1], Frank Pieterse[1], Edgar A. Chavarro-Carrero[1], Marie-Emma Denarie[1], Si Qin[1], Iris Kappers[2], André Fleißner[3] and Jan A.L. van Kan[1]

[1] *Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands.*

[2] *Wageningen University, Laboratory of Plant Physiology, Wageningen, The Netherlands.*

[3] *Technische Universität Braunschweig, Institute of Genetics, Braunschweig, Germany.*

α-Tomatine, a steroidal glycoalkaloid saponin, is abundant in vegetative tissues of tomato and has antibiotic activities. Secretion of glycosyl hydrolases (GH) to enzymatically degrade α-tomatine into less toxic products is the only mechanism reported in tomato pathogens so far.

A unique tomatinase activity (β-xylosidase) in *Botrytis cinerea* has been reported more than 20 years ago, however, attempts to clone this gene based on homology to other tomatinase genes failed. In this study, we identified α-tomatine-inducible genes in isolate B05.10 by RNAseq. We observed strong induction of genes encoding a GH from the GH43 family, two glycosyl transferases (GT) from the GT28 family as well as membrane proteins including one ABC transporter and multiple RTA1-like proteins. In addition, the genome sequencing of *B. cinerea* isolate M3a, which is unable to degrade α-tomatine, revealed a missing locus compared with B05.10 comprising the *BcGH43* and one *BcGT28a* due to the insertion of a transposable element.

Recombinant BcGH43 protein exhibited *in vitro* tomatinase activity through hydrolysis of the terminal xyloside of α-tomatine. The B05.10 knockout (KO) mutant of BcGH43 lost tomatinase activity, became more sensitive to α-tomatine and less virulent on tomato. Moreover, the overexpression (OE) of either the *BcGH43* or the other two types of tomatinase genes from *Septoria lycopersici* (GH3) and *Cladosporium fulvum* (GH10) separately in M3a conferred tomatinase activities, and restored the virulence on tomato.

The deletion of the *BcGT28* (absent in M3a) in B05.10 reduced its virulence on tomato but did not impact α-tomatine sensitivity. However, OE of *BcGT28* in M3a led to elevated resistance to α-tomatine and increased virulence on tomato. Upon α-tomatine treatment, the GT28a-GFP fusion protein was recruited from cytosol to the membranes of hyphal tips (abundant in ergosterol) indicating the importance of membrane modification in resistance to α-tomatine and virulence on tomato. We are in the process of analysing glycosylated fungal membrane compounds in response to α-tomatine in wild-type B05.10 with reduced/abolished glycosylation pattern in the *BcGT28a* single and double KO mutants to elucidate the mechanism underlying membrane modification conferred by BcGT28a. Besides, KO mutants and OE transformants of other α-tomatine-inducible genes in B05.10 and M3a have been generated and are currently under characterization.

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SESSION IV: HOST-PATHOGEN INTERACTION – VIRULENCE FACTORS

POSTERS

Transcriptomic analysis of the interaction between *Botrytis cinerea* and *Trichoderma atroviride* using a GRN approximation.

Consuelo Olivares-Yañez[1,2]*, Evelyn Sanchez[2,3]*, Gabriel Perez[1,2], Pamela Camejo[2],

Aldo Seguel-Avello[2,4], Luis Larrondo[2,4], Elena Vidal[2,3] and Paulo Canessa[1,2]

[1] *Laboratorio de Genética de Hongos. Centro de Biotecnología Vegetal. Facultad de Ciencias de la Vida. Universidad Andrés Bello. Santiago.Chile.*

[2] *ANID – Millennium Science Initiative Program - Millennium Institute for Integrative Biology (iBio). Santiago. Chile.*

[3] *Centro de Genómica y Bioinformática. Facultad de Ciencias. Universidad Mayor. Santiago Chile*

[4] *Fungal Genetics and Synthetic Biology Laboratory. Genetics and Microbiology Department. Biological Science Faculty. Pontificia Universidad Católica. Santiago. Chile.*

The use of microorganisms to control plant diseases, has emerged as a promising alternative to the use of chemical pesticides. *T. atroviride* is one of the most used biocontroller fungi because of its ability to attack a wide range of plant pathogens, including *B. cinerea.*

B. cinerea is a necrotrophic fungus capable of infecting more than 1000 plant species, causing massive economic losses. Nowadays, the use of biocontroller organisms to contain the infection generated by this fungus is not enough as they must be applied with a mixture of pesticides. Although Trichoderma's use in agricultural fields has been growing steadily in recent years, the interaction of these two organisms remains mainly underexplored.

To generate new and more efficient ways of biocontrol of this phytopathogen using *Trichoderma*, it has become relevant to understand the molecular dialogs during their interaction better. We generated a manually curated transcription factor database (TFDB) for *T. atroviride* and B*. cinerea* to develop transcriptional gene regulatory network models (GRN). Using InterProScan and a hmmsearch scouting, protein sequences were inspected and then annotated employing BLAST2GO and FungiFun software. After a manual curation procedure, 561 and 471 sequences were considered true TFs for *T. atroviride* and *B. cinerea*, respectively. After that, for 75.4% and 79.6% TFs in *T. atroviride* and *B. cinerea*, we established their respective DNA binding preference employing CisBP-DB. Finally, TF-target gene interactions were predicted with FIMO and subsequently refined using the network inference algorithm GENIE3.

After dual RNA-seq analysis of the Trichoderma-Botrytis confrontation, the obtained GRN models were used to build context-specific networks of the interaction. The transcriptomic data of the confrontation assay showed a different response regarding the total number of Differentially Expressed Genes (DEG) in each fungus, being significantly stronger in the biocontroller. Network analysis revealed different gene expression responses associated with a mycoparasitic behavior in *Trichoderma* and defense responses in *Botrytis*. Indeed, we were able to identify a set of 22 DEG peptidases in *Trichoderma* regulated by 4 TFs. In the case of *Botrytis*, most of the genes were upregulated and related to oxidative stress responses. We are currently analyzing the impact of some of these *B. cinerea* TFs in the interaction by generating their respective loss-of-function mutants.

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Snf1 kinase differentially regulates *Botrytis cinerea* pathogenicity according to the plant host

Szabina Lengyel[1,2], Christine Rascle[2], Nathalie Poussereau[2], Christophe Bruel[2], Luca Sella[1], Mathias Choquer[2] and Francesco Favaron[1]

[1]*Department of Land, Environment, Agriculture and Forestry (TESAF),*

University of Padova, Viale dell'Università, 16, 35020 Legnaro, Italy.

[2]*Univ Lyon, Université Claude Bernard Lyon 1, CNRS, Bayer SAS, INSA-Lyon, UMR5240, Microbiologie, Adaptation et Pathogénie, 14 Impasse Pierre Baizet, F-69263 Lyon, France.*

The Snf1 kinase of the glucose signaling pathway controls the response to nutritional and environmental stresses. In phytopathogenic fungi, Snf1 acts as a global activator of plant cell wall degrading enzymes that are major virulence factors for plant colonization. To characterize its role in the virulence of the necrotrophic fungus *Botrytis cinerea*, two independent deletion mutants of the *Bcsnf1* gene were obtained and analyzed. Virulence of the *Δsnf1* mutants was reduced by 59% on a host with acidic pH (apple fruit) and up to 89% on hosts with neutral pH (cucumber cotyledon and French bean leaf). *In vitro*, *Δsnf1* mutants grew slower than the wild type strain at both pH 5 and 7, with a reduction of 20-80% in simple sugars, polysaccharides, and lipidic carbon sources, and these defects were amplified at pH 7. A two-fold reduction in secretion of xylanase activities was observed consequently to the *Bcsnf1* gene deletion. Moreover, *Δsnf1* mutants were altered in their ability to control ambient pH. Finally, *Δsnf1* mutants were impaired in asexual sporulation and did not produce macroconidia. These results confirm the importance of *BcSnf1* in pathogenicity, nutrition, and conidiation, and suggest a role in pH regulation for this global regulator in filamentous fungi.

Lengyel *et al*. 2022, *Microorganisms*, 10, 444.

Functional characterization of a *Botrytis cinerea* protease involved in the infection process.

Antoine Davière[1,2], Marie-Christine Soulié[1,3] and Mathilde Fagard[1].

[1]*Institut Jean-Pierre Bourgin, INRAE, AgroParisTech, Université Paris-Saclay, 78000 Versailles, France*

[2]*Doctoral school of Plant Science, Université Paris-Saclay, 91405, Orsay, France*

[3]*Sorbonne Universités, UPMC Université Paris 06, UFR 927, 75005 Paris, France.*

Botrytis cinerea is a nectrotrophic plant pathogen that leads to important economic losses in many crops. This fungus has a large arsenal of virulence factors such as cell wall degrading enzymes, toxins, reactive oxygen species production, small RNA production and proteases. Furthermore, *B. cinerea* is known to acidify the medium by secreting succinic and citric acids as well as oxalate to enhance enzymatic activities during the infection process. Recently, a new class of proteases that work at a very low pH has been discovered. These glutamic proteases, almost entirely restricted to filamentous fungi, comprise 3 homologs in *B. cinerea*. Roland et al showed that *BcACP1* encodes a glutamic protease that is regulated *in vitro* by nitrogen and sulfur availability and pH (2009, *Microbiology*, 155(6), 2097-2105). Other works point out the facts that this protease is present in the early secretome of botrytis and preferentially produced from infection cushions, known for their role in plant-cell wall penetration. Our team demonstrated that the expression of the *BcACP1* gene is up-regulated on infected plants grown with high nitrate supply, which are more susceptible to the pathogen in comparison with low nitrate supply (Soulie et al. 2020, *Molecular Plant Pathology*, 21, 1436–1450). This induction cannot be explained by nutrient availability, indicating a fine regulation of this effector by the plant physiological state. As the protease is expected to localize in the apoplastic space during the early stage of the interaction, we are searching for specific targets and regulators in this compartment using proteomic and metabolomic analysis on apoplast washing fluid (AWF) extracts. On the other hand, we are analyzing the ability of BcACP1, purified in *E. coli,* to degrade plant apoplastic proteins and to activate or repress plant defense reactions on different host species. Functional analysis reveals that neither *BcACP2* nor *BcACP3* are indispensable for pathogenicity and development. Since *BcACP2* transcription is up-regulated in the *ΔBcacp1* mutant, analysis of the double mutants is under process and might reveal new clues about the role of this protease family. Furthermore, this work will lead us to the description of the infected AWF proteome and metabolome at the beginning of the infectious process. This analysis might enable us to identify novel plant compounds and effectors involved in apoplastic immunity as well as early secreted effectors of the fungus.

Adressing redundant roles of phytotoxic compounds of *B. cinerea* for necrotrophic infection by multi-k.o. mutagenesis

Thomas Leisen[1], Nassim Safari[1], Patrick Pattar[1], Frederik Sommer[1], Michael Schroda[1], Ivonne Suárez[2], Isidro Collado[2], David Scheuring[1], Sophie Eisele[1], Tabea Lang[1] and Matthias Hahn[1]

[1]Department of Biology, Technische Universität Kaiserslautern, 67663 Kaiserslautern, Germany. [2]Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Cádiz, Spain.

Botrytis cinerea is a necrotrophic pathogen infecting a wide range of host plants. During invasion, it quickly kills host cells and colonizes dead tissue. Factors that contribute to this lifestyle include secretion of CWDE, phytotoxic proteins and metabolites, tissue acidification and activation of PTI-related defence. However, it is not yet known how these activities lead to cell death in host tissue and successful invasion by the pathogen. We have established a high-efficiency CRISPR/Cas9 protocol that enabled us to generate multiple mutants lacking most of the currently known phytotoxic metabolites and proteins of *B. cinerea* (Leisen et al., 2020, PLoS Pathogens, https://doi.org/10.1371/journal.ppat.1008326; Leisen et al., 2022, PLoS Pathogens, https://doi.org/10.1371/journal.ppat.1010367). A series of 12-18-fold knockouts were constructed and characterized, in genes several of which have previously described to be important for infection. Genome sequencing confirmed the deletions and revealed only few off-target mutations, and MS/MS analysis of the secretomes produced by the mutants on host leaves verified the loss of the deleted CDIPs. The mutants showed generally decreased virulence with increasing numbers of deleted genes, but the effects of the deletions were dependent on the type infected host tissue. All multiple mutants retained their ability to form necrotic lesions, and their secretomes showed remaining phytotoxic activity. While searching for and deleting further CDIPs, we are also investigating the role of activation of plant immune receptors by these proteins for cell-death induction and necrotrophic pathogenesis. Starting with infections of plant mutants or silenced tissues lacking the coreceptors of pattern recognition receptor proteins (PRRs), BAK1 and SOBIR1, failed to reveal major differences to WT plants in susceptibility against *B. cinerea* WT and 12x mutants, providing no clue yet about the way the fungus manipulates host defence pathways. Our data document one of the first systematic approaches to address functional redundancy of virulence factors of a pathogenic fungus, and the apparent absence of single major virulence proteins in *B. cinerea*.

Effector proteins of *Botrytis elliptica* as tools for resistance breeding in lily against fire blight disease

Michele Malvestiti[1], Richard Immink[2], Paul Arens[3] and Jan van Kan[1]

[1]*Wageningen UR, Laboratories of Phytopathology, Wageningen, The Netherlands*

[2]*Wageningen UR, Department of Bioscience, Wageningen, The Netherlands*

[3]*Wageningen UR, Department of Plant Breeding, Wageningen, The Netherlands*

Fire blight represents a widespread disease in lily and is caused by the host specific Ascomycete *Botrytis elliptica*. As a necrotrophic fungus *B. elliptica* acquires nutrients from dead plant tissue. Therefore, induction of Programmed Cell Death (PCD) is a key step for infection and is achieved by the activity of secreted effector proteins. We hypothesise that susceptibility to fire blight in a lily cultivar correlates to effector sensitivity and that effectors quantitatively contribute to virulence. This research aims to identify and characterize *B. elliptica* effectors and use them to screen the lily germplasm to select for plants which display increased fire blight resistance. Via disease assays, we tested twelve different *B. elliptica* isolates on eighteen lily cultivars (Malvestiti et al. 2021, FiPS, 12, 660337). By measuring lesion size we observed variation in symptom severity in the different isolate-cultivar combinations. Similar variation was also found in flowers. Interestingly, flowers showed faster expanding lesions compared to leaves. The variation in virulence among the tested isolates allowed to select the most aggressive one to study the effectors. By growing the fungus in liquid media, we collected all secreted compounds in a culture filtrate (CF). The CF was subjected to ion exchange chromatography to obtain purified protein fractions. After leaf infiltration with the CF and the single fractions we observed variation in sensitivity in the diverse lilies. Not all fractions showed PCD inducing activity. Those lilies which showed severe symptoms in the disease assay were the same lilies which showed high sensitivity to the infiltrated samples. The protein profile of active fractions was integrated with genomic and transcriptomic data to obtain a list of candidate effector genes. Candidate genes were expressed in yeast to obtain purified effector proteins which can be used to screen the lily germplasm. Their importance for fungal virulence will be validated via targeted gene replacement. Finally, by investigating effectors structure, activity and protein-protein interactions, we can identify effector targets in lily. This will give insights in the mechanism of PCD induction and allow the identification of susceptibility genes.

Effector repertoire analysis of the necrotrophic fungal pathogens *Botrytis squamosa* and *Botrytis elliptica*

Michele Malvestiti, Maikel Steentjes and Jan van Kan

Wageningen UR, Laboratories of Phytopathology, Wageningen, The Netherlands

Fungal plant pathogens secrete effector proteins that manipulate the host in order to facilitate colonization. Necrotrophs have evolved specialized effector proteins that actively induce plant cell death by co-opting the programmed cell death machinery of the host. In this study we aim to identify cell death-inducing effectors of host-specific Botrytis species. Besides the broad host range pathogen B. cinerea, most other species within the genus Botrytis are restricted to a single host species or a group of closely related hosts. Here, we focus on Botrytis squamosa and B. elliptica which host specific pathogens of onion (Allium cepa) and lily (Lilium spp.), respectively. Despite their different hosts, the two fungal species are each other's closest relatives. For both species, we used a genome-wide effector prediction to obtain both shared and species-specific candidate effector proteins. RNAseq analysis during infection of onion and lily, respectively, revealed which effectors are expressed during the early stages of host colonization. To assess the potential effector activity, we produced culture filtrates from B. squamosa and B. elliptica grown in liquid culture. Both culture filtrates induced strong cell death responses upon infiltration in host tissue. Mass spectrometry analysis of the secretome revealed the identity of effector proteins present in the cell death-inducing culture filtrate. To evaluate if the effectors had been subject to diversifying selection, the genetic diversity of protein coding sequences was compared among different isolates of both B. squamosa and B. elliptica. The identification of shared and species-specific effector proteins of B. squamosa and B. elliptica provides insights in their contribution to host specificity of Botrytis species.

Role of a LysM-domain containing protein in the virulence of the necrotrophic plant-pathogen fungus *Botrytis cinerea*

Mélanie Crumière[1], Amélie De Vallée[1], Christine Rascle[1], Jan van Kan[2], Christophe Bruel[1], Nathalie Poussereau[1] and Mathias Choquer[1]

[1] Univ Lyon, Université Claude Bernard Lyon 1, CNRS, Bayer SAS, INSA-Lyon, UMR5240, Microbiologie, Adaptation et Pathogénie, 14 Impasse Pierre Baizet, F-69263 Lyon, France.

[2] Wageningen University & Research, Laboratory of Phytopathology, Wageningen, Netherlands

Because of its immunogenicity, the polysaccharide chitin that is part of the fungal cell wall plays an important role in the molecular interaction between plants and their fungal parasites. To initiate successful infection, fungi have developed strategies to avoid the perception of chitin fragments from their cell wall by their host plants. One of these strategies involves secretion of proteins displaying LysM domains able to bind fragments of chitin. In biotrophic fungi, LysM-domain containing proteins interact with chitin to prevent its degradation by plant chitinases or to interfere in the recognition of chitin fragments by the plant (Akcapinar *et al.* 2015, *Current Genetics*, 61, 103-113). Such mechanisms have never been considered for necrotrophic fungi, but recent results obtained in the necrotrophic model *Botrytis cinerea* suggest a role for a protein containing a single LysM domain during the plant-fungus interaction. Transcriptional induction of the gene encoding BcLysM1 was observed during the differentiation of mature infection cushions, structures dedicated to the penetration and necrosis of the host (Choquer *et al.* 2021, *Environmental Microbiology*, 23, 2293-2314) and at early stages of infection on bean leaves. The protein BcLysM1 was produced in *Pichia pastoris* and its capacity to bind chitin was confirmed. Localization of the protein into *Botrytis* cell wall was also verified. A *BcLysM1* gene deletion mutant was constructed and a delay in necrosis induction was observed when the fungus was inoculated with mycelium plugs on bean leaves. The role of the BcLysM1 protein in the plant immunity response was analysed and several genes implicated in host response were shown to be upregulated when bean leaves were inoculated with the deleted strain. Moreover, the deletion strain showed a reduced adhesion on artificial surface and on plant leaves. This study is the first describing a LysM-domain containing protein in a necrotrophic ascomycete, and provides a new hypothesis concerning the role of these fungal effectors in the adhesion of the fungus onto the plant host.

Cross-kingdom RNA interference in early phases of the *Botrytis cinerea*-tomato interaction

Si Qin[1,*], Javier Veloso[1,2*], Guido Puccetti[1], Tim Bosman[1], Mirna Baak[3], Britt Boogmans[1], Xiaoqian Shi-Kunne[1], Sandra Smit[3], Thomas Leisen[4], Matthias Hahn[4] and Jan van Kan[1]

[1]*Laboratory of Phytopathology, Wageningen University, The Netherlands.*

[2]*FISAPLANT, University of A Coruña, Spain.*

[3] *Bioinformatics Group, Wageningen University, The Netherlands.*

[4]*Department of Biology, University of Kaiserslautern, Germany.*

**These authors contributed equally to this work*

Botrytis cinerea was previously reported to use small RNAs (sRNAs) as effector molecules capable of interfering with the host immune response. Conversely, a host plant produces sRNAs that may interfere with the infection mechanism of the intruder. We used high-throughput sequencing to identify the small RNAs produced by *B. cinerea* and *Solanum lycopersicum* (tomato) during their interaction and to examine the expression profile of their (predicted) mRNA targets in the other organism. Most of the ~28,000 sRNAs (with the length of 20-24 nt) produced by *B. cinerea* originated from ribosomal RNA (47% of the total) and transposable element regions (33% of the total). About a quarter of the sRNAs produced by *B. cinerea* were predicted to target a total of 3185 mRNAs in tomato. Of the predicted tomato target genes, 56 were indeed transcriptionally down-regulated during the early phase of infection.

Several experiments were performed to study a causal relation between the production of sRNAs and the down-regulation of predicted target genes in the other organism. On one hand, we generated a fungal mutant in an effector gene (*Bcspl*1) which was predicted to be targeted for silencing by a tomato sRNA. The predicted target sequence for the sRNA was replaced by synonymous substitutions, however, the *Bcspl1* transcript in the mutant did not show any evasion of downregulation. On the other hand, we generated *B. cinerea* mutants in which a transposon region that is the source of ~10% of the fungal sRNAs was deleted, as well as mutants in which both Dicer-like genes (*Bcdcl1* and *Bcdcl2*) were deleted. Neither of these mutants was significantly reduced in virulence on tomato or several other tested plant species, despite a 97% reduction of sRNA production in *ΔBcdcl1ΔBcdcl2* double mutants.

I will present the concept and results in this study that illustrate the complexity and many possible variables that operate in the cross-kingdom RNA warfare between *B. cinerea* and its host plants.

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Generation and characterization of multiple B. *cinerea* mutants reveal a high redundancy of phytotoxic proteins and metabolites for necrotrophic infection

Nassim Safari[1], Thomas Leisen[1], Patrick Pattar[1], Tabea Lang[1], Sophie Eisele[1], Ivonne Suárez[2], Isidro Collado[2], David Scheuring[1] and Matthias Hahn[1]

[1]Department of Biology, Technische Universität Kaiserslautern, 67663 Kaiserslautern, Germany.

[2]Departamento de Química Orgánica, Facultad de Ciencias, Universidad de Cádiz, Cádiz, Spain.

The necrotrophic ascomycete *Botrytis cinerea* characterized by a very wide range of host plants. Mechanisms that contribute to host killing include the secretion of CWDE and the release of phytotoxic proteins and metabolites during invasion, resulting in a hypersensitivity-like plant cell death and successful invasion by the pathogen. We have developed a highly efficient CRISPR/Cas9 protocol to generate marker-free, homokaryotic mutants, which allowed us to perform serial eliminations of most of the currently known phytotoxic metabolites and proteins in single *B. cinerea* mutants (Leisen *et al.*, 2020, *PLoS Pathogens*, https://doi.org/10.1371/journal.ppat.1008326). By this means, we have constructed and characterized strains with a series of up to 12- and 18-fold knockouts of genes, several of which have previously shown to be important, such as Spl1, Nep1/2, Xyn11A, Xyg1, Hip1, IEB1, Xyl1, Gs1, CDI, PG1/PG2, and the phytotoxic metabolites botrydial and botcinin (Leisen *et al.*, 2022, *PLoS Pathogens*, https://doi.org/10.1371/journal.ppat.1010367). Phenotypic analyis of these mutants revealed decreased virulence with increasing numbers of deleted genes. The effects of the deletions were dependent on the infected host tissue and differed between leaves of beans, tomatoes, maize, tobacco, Arabidopsis; apple fruit. Multiple mutants were still able to form necrotic lesions, and their secretomes had substantial remaining phytotoxic activity. We have also tested the role of activation of plant pattern recognition receptor (PRR) coreceptors BAK1 and SOBIR1. Infection of tobacco and Arabidopsis *sobir1* and *bak1* mutants or silenced tisssue lacking a coreceptor of LRR-RP receptor proteins did not reveal major differences to WT plants in susceptibility against *B. cinerea* WT and 12x mutants ; therefore it remains unclear how the fungus manipulates host defence pathways. Experiments are underway to eliminate the remaining known and yet to-be-discovered phytotoxic proteins, in order to generate multiple *B. cinerea* mutants that show normal vegetative growth and differentiation but that are deprived in their ability to kill plant cells.

Effector proteins of *Botrytis squamosa* have cell death-inducing activity but are not essential for virulence

Maikel B.F. Steentjes[1], Andrea Lorena Herrera Valderrama[1], Claudio A. Valero Jiménez[1], Olga E. Scholten[2] and Jan A.L. van Kan[1]

[1] *Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands.*

[2] *Wageningen University, Plant Breeding, Wageningen, The Netherlands.*

Botrytis squamosa is the causal agent of onion leaf blight disease, one of the most destructive diseases in onion cultivation worldwide. The initial symptoms of onion leaf blight are characteristic small necrotic lesions that in a later stage can expand, leading to early leaf senescence. We aimed to identify effector proteins of *B. squamosa* that induce host cell death in *Allium cepa* (onion). Sensitivity of onion genotypes to the effector is hypothesized to be correlated with susceptibility to the fungus that is producing the effector. Conversely, insensitivity to the effector may confer (partial) resistance to *B. squamosa*. Such an effector protein can be used as a direct tool to screen onion germplasm for resistance in breeding programmes.

In this study, we used a combination of three approaches to identify effector proteins. First, we sequenced the genomes of *B. squamosa* and closely related sister species such as the lily pathogen *B. elliptica*. Using comparative genomics, we aimed to identify genes that are unique for *B. squamosa*. Second, we performed a transcriptome analysis of early infection timepoints of a *B. squamosa*-onion infection to determine which genes are highly expressed during plant colonization. For the third approach, we produced culture filtrates and purified them based on their cell death-inducing activity in onion. The bioactive fractions were analysed by mass spectrometry, resulting in a list with candidate effectors. By producing the candidate effector proteins, using the heterologous expression system *Pichia pastoris*, we were able to evaluate the cell death-inducing activity of individual effector proteins.

We have identified and evaluated the activity of multiple effectors. Both established effectors, such as Nep1 and Spl1 homologs found in *B. cinerea*, and novel effectors were identified. While some effectors induced cell death in a wide variety of tested plants, others did not trigger any plant response. To test the role of the effector proteins in virulence, *B. squamosa* knock-out mutants were made using the CRISPR-Cas9 method as developed for *B. cinerea*. Infection assays showed that none of the effectors tested so far, were essential for the virulence of *B. squamosa* on onion. Altogether, our results suggest that *B. squamosa* produces effector proteins that have cell death-inducing activity but are not essential for virulence.

Nitric oxide and the nitrilase coding gene family in *Botrytis cinerea*

Isabel Torres Quezada[1], Francisco Anta Fernández[2], Virginia Casado del Castillo[2], Óscar Lorenzo[1], José María Díaz Mínguez[2] and Ernesto Pérez-Benito[2]

[1]*Department of Botany and Plant Physiology, Institute for Agribiotechnology Research (CIALE), University of Salamanca, Salamanca, Spain.*

[2]*Department of Microbiology and Genetics, Institute for Agribiotechnology Research (CIALE), University of Salamanca, Salamanca, Spain.*

Nitrilases catalyse the hydrolysis of nitrile compounds to the corresponding carboxylic acid and ammonia. The presumed roles of these enzymes consist of cyanide detoxification, catabolism of natural nitriles, supply of nitrogen for growth, and production of indole-3-acetic acid under some specific conditions. Some fungal species are known to degrade cyanide through the action of a specialized subset of nitrilases called cyanide hydratases, which hydrolyze cyanide to formamide. The interest in nitrilases has been prompted by their ability to hydrolyze nitriles under ambient conditions and at mild pH values and to selectively hydrolyze cyano groups in presence of other functional groups. However, this activity was mainly examined with bacterial nitrilases while fungal nitrilases have been largely neglected for years (Martínková, 2009). Although the natural functions of nitrilases in fungi are not well understood, current evidence suggests that microbial nitrilases form part of an array of mechanisms that facilitate microbial colonization of plants, with putative roles in plant hormone synthesis, nitrogen utilization, catabolism of cyanogenic glycosides and glucosinolates, and the detoxification of nitriles and cyanide (Howden & Preston 2009). *Botrytis cinerea* has four nitrilase coding genes (*Bcin02g03010*, *Bcin14g00790*, *Bcin05g04960* and *Bcin12g06180*) of which two of them are highly induced after the addition of exogenous nitric oxide (NO). One of these nitrilase coding genes (*Bcin12g06180*) was particularly induced both in the wild type strain and a flavohemoglobin mutant, which constitutes the main NO detoxification method in the fungus. The aim of our study is the functional characterization of this heavily NO induced nitrilase through the generation of a knockout mutant via gene replacement strategy, and the elucidation of the role that NO plays in this complex and poorly studied process. Our preliminary results show that, although these mutants do not show alterations in the presence of exogenous NO, they are more sensitive to oxidative stress than the wild type strain and their growth is severely impeded by the addition of SNP and Potassium Ferricyanide (III), which may indicate that this enzyme is involved in cyanide detoxification.

References:

Martínková. 2019, *Fungal Biology Reviews*, 33: 149-157.

Howden & Preston. 2009, *Microbial biotechnology*, 2: 441-51.

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SESSION IV: HOST-PATHOGEN INTERACTION – PLANT DEFENSES

Chairs: Yigal Elad & François Lecompte

KEYNOTE LECTURE

A balancing act: How plant defenses and susceptibility factors impact the success of *Botrytis* infections

Barbara Blanco-Ulate[1]

[1]*Department of Plant Sciences, University of California, Davis, Davis, CA, United States*

The outcome of any plant-necrotroph interaction relies on the balance between the presence or induction of defenses and the contributions of susceptibility factors. Plant defense responses against necrotrophic fungi are multi-layered, involving the recognition of pathogen-associated molecular patterns (PAMPs) by pattern recognition receptors (PRRs), intracellular signaling through mitogen-activated protein (MAP) kinase cascades, induction of downstream defenses by coordinated activity of plant hormones, particularly ethylene and jasmonic acid, cell wall fortifications, and production of various secondary metabolites and antifungal proteins. Though induced defenses are heavily studied in plant immunity, the impact of preformed (or constitutive) defenses and susceptibility factors are less researched. Preformed defenses include structural barriers, such as the cell wall and cuticle, and the accumulation of secondary metabolites. In contrast, susceptibility factors can consist of the abundance of simple sugars and organic acids or the activity of host cell wall modifying proteins. For the past six years, my research team has studied the contributions of defenses and susceptibility factors to the success of *Botrytis* infections in fruit. *B. cinerea* displays different infection dynamics in fruit from those observed in vegetative tissues. Though both reproductive and vegetative tissues become more susceptible to *B. cinerea* during senescence, in fruit, a dramatic increase in susceptibility is observed before senescence during ripening. Using tomato as a model fruit host, we demonstrated that the ripening-associated susceptibility is best explained by a dominant role of susceptibility factors that increase during ripening which, coupled with a modest loss of preformed defenses, outweighs the efforts of the defense response in ripe fruit. The most significant contributor to fruit susceptibility is the disassembly of the plant cell wall-associated with fruit softening, given the importance of this structure as a physical barrier to fungal infection and a source of plant defense signals. We have also observed that *B. cinerea* can accelerate fruit ripening and cell wall disassembly when inoculated on unripe tomato fruit. The acceleration of ripening events in the host leads to the accumulation of susceptibility factors that facilitate disease development. Our work further suggests that targeting specific genes that drive susceptibility is a viable strategy to halt *B. cinerea* infection strategies and improve plant resistance against fungal disease.

Exploring plant genes that participate in the response to the phytotoxic metabolite botrydial produced by *Botrytis cinerea*

Si Qin[1], Sabine de Graauw[1], Haris Spyridis[1], Joost Keurentjes[2], Inmaculada Izquierdo[3], Isidro González Collado[3] and Jan A.L. van Kan[1]

[1] *Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands.*

[2] *Wageningen University, Laboratory of Genetics, Wageningen, The Netherlands.*

[3]*Universidad de Cádiz, Departamento de Química Orgánica, Cádiz, Spain.*

Botrydial (BOT) is one of the major phytotoxic metabolites produced by *Botrytis cinerea,* which can induce programmed cell death (PCD) in the host by interacting with plant components, and thereby facilitate fungal infection. Thus, the genes encoding plant components involved in the PCD pathways may serve as susceptibility (*S*) genes. We observed that the *Δbot2* mutant (Leisen et al. 2020, *PLoS pathogens*, *16*, e1008326), which does not synthesize BOT, was avirulent on the model plant *Arabidopsis thaliana* (Arabidopsis). This observation intrigued us to explore the underlying mechanisms of the plant-phytotoxin interaction and to further identify potential *S* genes to *B. cinerea*.

In this project, we applied genomic and genetic strategies to study the interactions between Arabidopsis components and BOT at molecular level. 1) We assessed the sensitivity to pure BOT of 359 Arabidopsis ecotypes belonging to a haplotype mapping population. Subsequently, we employed genome-wide association studies (GWAS) to pinpoint the allelic variations correlated with the plant sensitivity to BOT. The GWAS resulted in several loci in the Arabidopsis genome that are significantly associated with the phenotype. 36 candidate genes (including 12 cysteine-rich receptor-like protein/kinase genes) located in these loci were functionally validated using their corresponding Arabidopsis mutant lines with BOT sensitivity assay. 2) We crossed a BOT-sensitive Arabidopsis ecotype Col-0 with a relatively insensitive ecotype Uk-1, and subsequently assessed the BOT-sensitivity among the F1 and F2 populations. In order to identify genes that contribute to PCD triggered by BOT, some of the candidate genes identified GWAS were used as molecular markers to examine their correlations with BOT sensitivity in the F2 population. Interestingly, we found some of the candidate genes may function as positive or negative regulators in the PCD induced by BOT. I will present the results from both strategies and discuss the future perspectives of this study.

*The project is supported by China Scholarship Council.

Development of biomarkers for early *Botrytis cinerea* infections in strawberry fruit using multispectral imaging and volatile profiling approaches

Saskia D. Mesquida-Pesci[1], Eva Borras[2], Pedro Bello[3], Dilasha S. Shenaz[1], Cristina E. Davis[2], and Barbara Blanco-Ulate[1]

[1]*Department of Plant Sciences, University of California, Davis, Davis, CA, United States;*
[2]*Mechanical and Aerospace Engineering, University of California, Davis, Davis, CA, United States;*

[3]*Department of Plant Sciences, Seed Biotechnology Center, University of California, Davis, Davis, CA, United States*

Strawberries are an economically important soft fruit crop grown worldwide. Ripe strawberries are highly susceptible to gray mold caused by *Botrytis cinerea*, while unripe strawberries are resistant. After invading the receptacle of unripe fruit, *B. cinerea* enters a symptomless phase until ripening or favorable conditions promote the transition to the necrotrophic stage. Knowledge on how *B. cinerea* establishes a compatible interaction with its fruit host is limited. The lack of resistant strawberry cultivars and rapid development of fungicide resistance in *B. cinerea* call for fast, non-destructive methods to detect the presence of *B. cinerea* in strawberries before lesions are evident. We hypothesize that *B. cinerea* can manipulate host responses to promote disease, and that infections on strawberry induce changes in host secondary metabolites that can be detected by assessing changes in the light absorption profile of fruit surfaces through multispectral imaging (MSI) and by profiling volatile organic compounds (VOCs). We used a MSI system to compare the spectral profiles of *B. cinerea*-inoculated versus mock-inoculated fruit at various infection times, from 6 to 60 hours post inoculation (hpi). We determined that wavelengths in the visible and UV areas of the light spectrum allow for a distinct significant separation between fungal infection and wounding responses, and that reflectance profiles greatly differ between inoculated and mock fruit. In parallel, VOCs were sampled at similar time points (9 to 24 hpi) from healthy and inoculated strawberries. Partial least squares discriminant analyses of VOC profiles showed that infected samples clustered together at 9 hpi, as opposed to higher dispersion of mock samples, which points to a coordinated early host response to *B. cinerea* infection. We then performed a dual RNAseq study of strawberries inoculated with *B. cinerea* to confirm the induction of specific metabolic pathways during early infection and further validate the selection of MSI and VOC features as potential biomarkers. We are currently analyzing the RNAseq data. This project highlights the potential of combining both approaches for early detection of gray mold in postharvest scenarios to aid disease management and reduce produce losses. Furthermore, this research will generate knowledge of the early interactions of *B. cinerea* with strawberry fruit to define specific infection strategies used by the fungus and defense mechanisms deployed by the host.

Increased metabolic fluxes in tomato stems infected by *Botrytis cinerea*

Nathalie Lacrampe[1], Raphael Lugan[2], Philippe C. Nicot[3], Sophie Colombié[4]

and François Lecompte[1]

[1]INRAE, PSH, F-84914 Avignon, France.

[2]Avignon Université, UMR Qualisud, F-84916, France.

[3]INRAE, Pathologie végétale, F-84140 Montfavet, France.

[4]INRAE, UMR 1332 BFP, F-33883 Villenave d'Ornon, France

Metabolic reprogramming is part of the plant's defence response following infection by necrotrophic fungi. However, the nature and intensity of metabolic changes contributing to resistance are not well understood. Flux balance analysis (FBA) predicts metabolic flux distributions at steady state, using a constraint-based model relying on a stoichiometric network and optimization with a defined objective function, which hypothesizes optimal biological functioning in this network (Orth et al. 2010, *Nature Biotechnology*, 28, 245–248). FBA was successfully used to study plants physiological behaviour (Colombié *et al.*, 2015, *Plant Journal*, 81, 24-39), but has not yet been applied to plant-necrotrophic fungi interactions. We used nitrogen (N) nutrition to modulate the susceptibility of tomato stems to *Botrytis cinerea*, and examined at each N level the predicted metabolic fluxes in healthy stem tissues adjacent to lesions, in comparison with those of mock-inoculated plants.

The model encompasses 298 metabolic reactions, including the central metabolism, co-factors and redox homeostasis, and pathways towards main components of the biomass as well as main defence-associated secondary compounds. Quantitative metabolomics and other biochemical approaches were used to measure, before and 2, 4 and 7 days after inoculation, the concentrations of 46 "external" compounds, towards which fluxes were adjusted by derivation of their time-courses. These fluxes served as constraints in the model, which predicted the remaining 242 "internal" fluxes as well as C and N imports and gaseous exchanges, under the additional assumptions of internal steady state and the minimization of fluxes as an objective function. Radial growth was assessed by the measurement of stem diameters at different time points.

The model predicted increased fluxes in several pathways of *Botrytis*-inoculated plants in comparison to mock-inoculated ones, whatever the N level. This was driven by an enhanced growth after *B. cinerea* inoculation, which surprisingly increased with N deficit. When the fluxes were normalized according to C input, the model better highlighted distinct metabolic profiles according to the N regime, which could, at least partly, explain the observed range of susceptibilities. This approach provides new insights for the comprehension of resistance to *B. cinerea* in tomato stems, and its link with N availability.

Cytokinin regulates energy utilization in *Botrytis cinerea*

Gautam Anand, Rupali Gupta and Maya Bar

Department of Plant Pathology and Weed Research, ARO, Volcani Institute, Rishon LeZion 7505101, Israel.

The plant hormone cytokinin (CK) is an important developmental regulator, promoting morphogenesis and delaying senescence. Previous work by us and others has demonstrated that CKs also mediate plant immunity and disease resistance. Some phytopathogens have been reported to secrete CKs, and may manipulate CK signaling to regulate the host cell cycle and nutrient allocation, to improve their pathogenic abilities. In a recent work, we demonstrated that CK directly inhibits the growth, development, and virulence of fungal phytopathogens, by down regulating the cell cycle and reducing cytoskeleton organization and cellular trafficking in the fungus (Gupta et al., mBio 2021, 12:5, doi:10.1128/mbio.03068-20). Here, focusing on *Botrytis cinerea (Bc)*, **we report that CK possesses a dual role in fungal biology, with role prioritization being based on nutrient availability**. In a nutrient rich environment, CK strongly inhibited *Bc* growth and de-regulated cytoskeleton organization. This effect diminished as nutrient availability decreased. In its second role, we show using biochemical assays and transgenic redox sensitive fungal lines, that CK can promote glycolysis and energy consumption in *Bc*, both *in vitro* and *in planta*. In contrast with the inhibitory effects of CK, Glycolysis and increased oxidation mediated by CK were stronger with waning nutrient availability. Transcriptomic data further supports our findings, demonstrating significant upregulation to glycolysis, oxidative phosphorylation, and sucrose metabolism, upon CK treatment. Thus, the effect of CK in fungal biology depends on energy status. The metabolic effects of CK on the fungus likely reflect the role of plant CK during early infection by necrotrophic pathogens, which are reported to have an initial, short biotrophic phase. In addition to the plant producing CK during its interaction with the pathogen for defense priming and pathogen growth inhibition, the pathogen likely exploits this increased CK to boost its metabolism and energy production, in preparation for the necrotrophic phase of the infection. Thus, the role of CK in controlling senescence can be exploited by diverse fungal phytopathogens.

Using biomimetics to uncover the role of microstructure in Botrytis-plant interaction

Tali Sayas[1], Haguy Alon[1,2], Helen Rombach[3], Helena Vitoshkin[4], Lavanya Gunamalai[5], Carmit Ziv[5] and Maya Kleiman[1]

[1] *Plant Sciences Institute, Agricultural Research Organization, Rishon Lezion, Israel ,*

[2]*Inter-Faculty Graduate Biotechnology Program, The Hebrew University of Jerusalem, Rehovot, Israel*

[3]*Department of Agriculture and Horticulture, Humboldt University Zu Berlin, Berlin, Germany*

[4]*Institute of Agricultural Engineering, Agricultural Research Organization, Rishon LeZion, Israel*

[5]*Institute of Postharvest and Food Science, Agricultural Research Organization, Rishon LeZion, Israel*

The first point of contact in the interaction between plants and microorganisms is the plant surface. This interaction is composed of two components: the physical one (surface microstructure) and the chemical/molecular one (molecular signals expressed on the surface). The surface microstructure can be viewed as one more form of signaling affecting the microorganism behavior. When studying plant-microorganism interaction using the natural plant, it is impossible to separate those two components as they are entangled within the biological system. Hence, to study the surface effect, new tools need to be built.

Biomimetics is a field combining chemistry and material sciences to imitate biological systems. Natural systems resolve problems through structural solutions, particularly, microstructures. The structural solutions achieved by nature have fascinated researchers over the years, encouraging them to seek synthetic mimics. One example for microstructure mimic comes from the lotus leaf, known for its 'lotus effect' - self-cleaning properties. The lotus leaf served as a template for microstructure biomimetics, to generate self-cleaning synthetic materials.

We are building synthetic, biomimetic systems to test how leaf microstructure affects the behavior of different plant pathogens, specifically botrytis. We are using tomato, as a host plant for botrytis. We found that conidia distribution upon the leaf surface is dependent on leaf microstructure. We have also found that the microstructure changes conidia germination rate as well as colony spread. Additionally, we are using biomimetic to develop new surfaces with botrytis repellent properties, based on the natural microstructures that enable an easier wash of conidia from surfaces.

SESSION IV: HOST-PATHOGEN INTERACTION – PLANT DEFENSES

POSTERS

Evaluation of virulence of *Botrytis* species associated to legumes

Elīna Brauna-Morževska[1], Irina Petrova[1], Biruta Bankina[1], Jānis Kaņeps[1], Gunita Bimšteine[1], Ingrīda Neusa-Luca[1], Ance Roga[2], Dāvids Fridmanis[2] and Frederick L. Stoddard[3]

[1]*Institute of Soil and Plant Sciences, Latvia University of Life Sciences and Technologies, Jelgava, Latvia.*

[2]*Latvian Biomedical Research and Study Centre, Riga, Latvia.*

[3]*University of Helsinki, Helsinki, Finland*

Botrytis genus fungi are significant pathogens of legumes. Well known species *B. cinerea*, *B. fabae* and during last decade discovered *B. fabiopsis* and *B. pseudocinerea* cause chocolate spot of beans and grey mould of many legume crops. Recently, many new *Botrytis* species were discovered using molecular methods, potentially pathogenic to legume crops (Brauna-Morževska *et al*. 2019, Research for rural development, 2, 63-69). The aim of this study was to evaluate *in vitro* virulence of *Botrytis* spp., isolated from different legume crops. In the period of 2014-2019, 278 isolates of *Botrytis* were obtained and selected by morphological traits from infected legumes in Latvia. A phylogenetic analysis was made by amplification of regions of three nuclear DNA gene combinations: RPB2, HSP60, and G3PDH. Six *Botrytis* spp. were identified on species level and five unknown *Botrytis* isolates were found. 28 representative isolates were selected for virulence testing on faba bean, field pea, lupin and soybean leaves *in vitro* by 5 mm mycelium-agar plugs. The diameter of the formed lesions under the inoculated plug was crosswise measured once a day, the experiments were performed twice with four replicates. Isolates that caused more than 5 mm lesions were defined as pathogenic. Virulence of *Botrytis* spp. was analysed by one-way ANOVA. Six *Botrytis* spp. were isolated from different legumes: *B. cinerea*, *B. fabae*, *B. pseudocinerea*, *B. fabiopsis*, *B. euroamericana* and *B. medusae*, as well five isolates that don't belong to any known *Botrytis* species. The virulence was significantly dependent on the *Botrytis* species and particular isolates. On peas were pathogenic *B. cinerea*, *B. pseudocinerea*, *B. fabiopsis*, *B. euroamericana* and *B. medusae*, but the most virulent were two unknown isolates. On lupin leaves most virulent were same two unknown isolates and *B. medusae*. *B. cinerea* and *B. pseudocinerea* caused pathogenic lesions. Only one unknown isolate was pathogenic on soybean leaves. On faba beans *B. fabae* as well as one unknown isolate were pathogenic. High variability was observed within the *Botrytis* species. Beyond to a known *Botrytis* species, there are unidentified species that can be significant and harmful legume pathogens. Further research with species identification and more data is needed.

The research was supported by the project of the Latvian Council of Sciences No. LZP-2019/1-0034 "Pathogenicity and diversity of *Botrytis* spp. – important causal agents of legume diseases".

Eugenol, endogenous compound in the Baco blanc cultivar, may affect *Botrytis cinerea* development in grapevine

Xavier Hastoy[1], Magaly Farel[2], Marie-Claude Ségur[3], Gilles de Revel[1] and Marc Fermaud[4]

[1]*Université de Bordeaux, INRAE, Bordeaux INP, OENO, UMR 1366, ISVV, F-33140 Villenave d'Ornon, France.*

[2]*Agriculture et Territoires, Chambre d'Agriculture des Landes, F-400005, Mont-de-Marsan,*

[3]*Bureau National Interprofessionel de l'Armagnac, F-32800 Eauze,*

[4]*INRAE, UMR SAVE, UMT SEVEN, Bordeaux Science Agro, ISVV, F-33882, Villenave d'Ornon, France.*

During European phylloxera crisis (1898), François Baco created a complex hybrid vine Baco blanc (*Vitis vinifera* x *Vitis riparia* x *Vitis labrusca*). This new cultivar was phytopathogen tolerant and productive of white wine intended for distillation of Armagnac spirits **(Baco, 1925).** Recent study on the cultivar has shown its atypical chemical profile with higher eugenol (well-known antiseptic molecule) content than in *V.vinifera* vines **(Franc et al., 2022)**. In 2006, professionals selected various individuals in Armagnac vineyards and created a conservatory showing variability within the Baco blanc cultivar (Chambre Agriculture Landes). This variability allowed us to raise the following questions about links: i) between Baco blanc intra-variability and susceptibility to the pathogen measured either in the vineyard or in biotest, ii) between a possible difference in susceptibility among Baco blanc clones and their eugenol content and/or other clonal features (agronomic, anatomo-physiological ones).

Botrytis epidemic development in vineyard was monitored on 6 Baco clones. At different phenological stages, agronomic indicators were evaluated: bunch compactness, bunch spatial distribution, leaf density, and - at 3 successive stages before harvest - grape maturation (Titratable Acidity, sugar concentration). Two bunch samplings were carried out for the chemical characterization of the berry skin, including eugenol *via* HS-SPME-GC-MS **(Franc *et al.,* 2022*)*** and for a biotest (berry artificial inoculation with 2 *Botrytis* strains leading to symptom assessments and final spores quantification).

The first results indicate, among the clones, a significant difference in free eugenol concentration in berry skin. In biotest, free eugenol may affect *Botrytis* pathogeny since a greater eugenol content in berry skin was associated with enhanced rotting, but with lower mycelium and/or sporulation development at the fruit surface. Symptom expression could have been then modulated according to the eugenol form: the bound one stimulating mycelium appearance and disfavouring sporulation whereas the free form would cause the opposite effect. In the vineyard, bunch compactness was confirmed as positively correlated with *Botrytis* epidemic severity, but negatively with the bound eugenol form. Thus eugenol, as a Baco blanc endogenous berry skin compound, may be associated to different pathological facies or severities in *Botrytis* epidemiology as shown in biotest and in the vineyard.

Cytolytic protein Nep1 from *Botrytis squamosa* induces cell death in onion leaves

Andrea Lorena Herrera Valderrama[1], Maikel B.F. Steentjes[1], Olga E. Scholten[2] and Jan A.L. van Kan[1]

[1] *Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands.*

[2] *Wageningen University, Plant Breeding, Wageningen, The Netherlands.*

Onion leaf blight disease is one of the most destructive diseases in onion cultivation worldwide. This problematic disease its caused by the fungus *Botrytis squamosa*, and its symptoms include smalls white lesions that can result in a complete leaf die-back causing significant yield loses. The effector repertoire of *B. squamosa* includes two proteins of the well-described class of Necrosis and Ethylene inducing Peptide 1 (NEP1)-like proteins (NLPs). To date, NLPs have been reported to possess cytolytic activity specifically on dicot plants but not on monocot plants. Typically, NLPs physically interacts with the terminal sugar residues of sphingolipids in the plant plasma membrane, which ultimately creates pores in the plant plasma membrane resulting in ion leakage and eventually cell death.

In this study, we addressed the biological function of *B. squamosa* Nep1 (*Bs*Nep1) and its role in the pathogenicity of *B. squamosa*. We produced the protein via the heterologous expression system *Pichia pastoris* and purified it using ion-exchange chromatography. *Bs*Nep1 activity was tested by infiltrating the protein in leaves of onion, as well as other monocot plant species. We observed a wide variation in *Bs*Nep1 sensitivity between different monocot plant species as well as between different onion genotypes. Analysis of their sphingolipids revealed that plants with similar sphingolipid contents can have differential sensitivities to *Bs*Nep1.

Our results show the functionality of cytolytic *Bs*Nep1 on monocot plant species and legitimize the presence of cytolytic NLPs in a monocot-specific pathogen. However, the sensitivity of monocots to *Bs*Nep1 cannot solely be explained by their plasma membrane sphingolipids content. Altogether, our findings contravene the dogma of dicot-specific cytolytic activity of NLPs, and might help to further elucidate plant sensitivity to cytolytic effector proteins.

Botrytis BcCrh1 working model and its application in plant protection

Yong Liang[1], Kai Bi[2] and Amir Sharon[1]

[1] *School of Plant Sciences and Food Security, Tel Aviv University, Tel Aviv, Israel.*

[2] *College of Life Science and Technology, Wuhan Polytechnic University, Wuhan City, China.*

The *B. cinerea* transglycosilase BcCrh1 is also a secreted cytoplasmic cell death inducing protein(CDIP). Similar to several other CDIPs, in addition to induction of cell death, BcCrh1 is recognized by the plant immune system and triggers defense responses. In a previous study we showed that a 35 amino acid (aa) BcCrh1-derived peptide is sufficient for induction of cell death. Here, we further characterized the protein, with the goal to examine the immune activation properties of BcCrh1 and its potential application to suppress infection. Using a structure analysis and a series of deletions and mutations we identified 3 amino acids within the 35 aa peptide that are necessary for induction of cell death. The mutant peptide retained immunogenicity, hence we could separate the induction of cell death from induction of plant defense. We further generated two derivatives of immune-triggering peptides, one extracellular and the second intracellular. Treatment of plants with these peptides induced local and systemic defense responses and reduced disease severity. Furthermore, *Arabidopsis thaliana* transgenic lines expressing the BcCrh1-derived peptides showed reduced sensitivity to *B. cinerea* infection. Our results show that induction of cell death and plant immunity are mediated by different parts of BcCrh1 that probably interact with separate plant receptors. We also demonstrated the potential use of BcCrh1 in increasing plant tolerance to *B. cinerea* infection.

Transcriptome analysis of compatible and incompatible interactions between *Botrytis cinerea* and tomato

Yaohua You[1], Xiaoqian Shi-Kunne[1], Yixuan Wang[2], Frank Pieterse[1], Danny Esselink[3] and Jan A.L. van Kan[1]

[1] *Wageningen University, Laboratory of Phytopathology, Wageningen, The Netherlands.*

[2] *Wageningen University, Laboratory of Plant Physiology, Wageningen, The Netherlands.*

[2] *Wageningen University, Laboratory of Plant breeding, Wageningen, The Netherlands.*

The necrotrophic fungal pathogen *Botrytis cinerea* can cause grey mould disease on many plants including cultivated tomato *Solanum lycopersicum*. Quantitative resistance to *B. cinerea* has been identified in the tomato wild relative *S. habrochaites* accession LYC4. Infection on LYC4 using inoculum containing 1000 spores/µL in Gamborg's B5 (GB5)-10 mM sucrose-10 mM phosphate medium can lead to high incidence of incompatible interaction, whereas compatible interaction was observed for infection on MM under the same infection condition or on LYC4 with increased sucrose concentration (50 mM). In this study an RNA sequencing (RNA-seq) approach was adopted to study the transcriptional reprogramming, both in the fungus and the plant, during the compatible and incompatible interactions between tomato and *B. cinerea*. Fungal gene expression remained similar during the compatible and incompatible interactions on LYC4 until 16 hpi, while at 24 hpi we observed in the incompatible interaction the induction of a large number of genes that were putatively under catabolite repression. The relief of catabolite repression at 24 hpi was substantiated in a fungal sample grown in 10 mM sucrose *in vitro*. *B. cinerea* displayed very different transcription patterns during infection on LYC4 as compared with MM infection, with the largest number of differentially expressed genes (2081) observed at 24 hpi. Moreover, *B. cinerea* genes such as *BcatrB* and *Bclcc2* involved in detoxification of plant antimicrobial compounds displayed substantially higher expression levels on LYC4 than on MM especially in the early infection stages (12 hpi). By contrast, α-tomatine-responsive genes in *B. cinerea*, for instance, *BcTom1* encoding tomatinase, exhibited delayed up-regulation on LYC4 in comparison with MM infection indicating delayed induction of plant cell death in LYC4. The delay in infection in LYC4 was also reflected by the later and weaker plant transcription response in LYC4 than in MM. Finally, we observed that LYC4 and MM differed in the expression of a large number of genes (>8000) even in the absence of *B. cinerea*.

*The project is supported by China Scholarship Council.

SESSION V: DISEASE MANAGEMENT I

Chairs: Gianfranco Romanazzi & Philippe Nicot

KEYNOTE LECTURE

A review of cultural practices for effective *Botrytis* management in New Zealand vineyards

Philip Elmer[1], Dion Mundy[2] and Peter Wood[3]

The New Zealand Institute for Plant and Food Research, [1] Ruakura, [2]Marlborough and [3]Havelock North, New Zealand.

Globally, there has been a significant shift in emphasis from synthetic fungicides to more integrated management of grey mould (*Botrytis*) caused by *Botrytis cinerea* in the last two decades. This change has been driven by several factors, including 1) the problems associated with the rapid emergence of resistance to commonly used synthetic fungicides; 2) increasing consumer demand for nil–low pesticide residues in food crops; 3) increasing demand for food production that does not negatively affect human and environmental health; 4) the continued growth of organic production systems; and 5) the greater availability of biofungicides.

Integrated *Botrytis* management is currently based on using a range of chemical and non-chemical practices to achieve effective disease control. This may include the strategic application of synthetic fungicides, use of cultural practices, the application of biological control agents and natural products, and fruit nutrition (e.g. calcium foliar sprays) to reduce host tissue susceptibility. If the present trends continue, future *Botrytis* management practices may be solely dependent on cultural practices and biofungicides.

The aim of this review is to examine the range of cultural control practices that have been scientifically validated in New Zealand vineyards since the 1980s, including 1) leaf removal and/or shoot thinning to improve air circulation and spray deposition; 2) under-vine mulching to reduce overwintering *B. cinerea* inoculum; 3) removal of necrotic floral tissues (post flowering) to reduce inoculum sites for early season establishment of *B. cinerea*; 4) optimal site selection and rootstocks and/or cover crops to devigorate vines; and 5) a relatively new tool, the mechanical thinning of bunches shortly after fruit set.

We explore advantages and disadvantages of each *Botrytis* control method. We conclude with predictions for future *Botrytis* management in vineyards. With even greater emphasis on reduced synthetic fungicide inputs, the cornerstone for *Botrytis* management in the future is likely to be an integration of cultural controls and selected clones and growing systems that provide the best physical environment to prevent disease, supported by integration with biofungicides with different modes of action.

Sclerotinia epidemic development and disease management in the upper Midwest U.S.

Martin I. Chilvers[1], Jill Check[1], Adam M. Byrne[1], Jaime F. Willbur[1], Younsuk Dong[2], Scott Bales[1] Mamadou L. Fall[3], Richard W. Webster[4] and Damon L. Smith[4]

[1]*Michigan State University, Department of Plant, Soil and Microbial Sciences, East Lansing, MI, United States*

[2]*Michigan State University, Department of Biosystems and Agricultural Engineering, East Lansing, MI, United States*

[3]*Agriculture and Agri-Food Canada, Saint-Jean-sur-Richelieu Research and Development Centre, Saint-Jean-sur-Richelieu, QC, Canada*

[4]*University of Wisconsin-Madison, Department of Plant Pathology, Madison, WI, United States*

Sclerotinia sclerotiorum the causal agent of Sclerotinia stem rot (SSR) is a significant threat to numerous crops in the north central United States including soybean, dry bean and potato. We utilize a systems approach to improve SSR disease management with an emphasis on soybean. From epidemiological approaches to identify environmental predictors of SSR epidemics and associated yield loss, spatiotemporal studies to examine the pattern of apothecia emergence as modulated by canopy closure and soil temperature, development of the app Sporecaster as an apothecia risk prediction tool, agronomic studies to investigate the role of soil fertility, row spacing and seeding rates on apothecia development and disease management, fungicide trials to optimize fungicide application timing and fungicide choice, and the screening of soybean varieties for SSR disease resistance to inform the selection of varieties by the farmer. We demonstrate that SSR disease management in soybean can be optimized by the selection of varieties that have a high level of partial resistance to SSR, the use of wide row spacing and lowered seeding rates, and the use of Sporecaster to examine SSR disease risk and inform fungicide application timing of an effective product when needed during flowering.

Peptaibol analogs as new effective fungicides against *Botrytis cinerea*

Angela Bolzonello[1], Isabella Busato[1], Arianna Panichi[1], Silvio Tundo[1], Marta De Zotti[2], Luca Sella[1] and Francesco Favaron[1]

[1]University of Padova, Department of Land, Environment, Agriculture and Forestry, Legnaro, Italy.[2]University of Padova, Department of Chemistry, Padova, Italy.

In the last decades the search for new effective and sustainable fungicides has gained much importance in the European political agenda. The well-known biocontrol agent *Trichoderma* spp. produces short hydrophobic non-ribosomal peptides, named peptaibols, with antibiotic properties given by their ability to permeabilize lipid bilayers such as the cell membrane. Specifically, the peptaibol trichogin produced by *T. longibrachiatum*, was used as a model to synthesize several water-soluble analogs (De Zotti et al., 2020 *International Journal of Molecular Sciences*, 21, 7521). These analogs present one or more substitutions of the glycine residues, which improve their water-solubility while maintaining their thermal and chemical stability. The aim of this study was to assess the fungicidal activity of trichogin analogs against *Botrytis cinerea.* With an *in vitro* screening, four peptides were identified as effective in inhibiting conidia germination at 15 µM concentration and the most effective peptide displayed a Minimal Inhibitory Concentration of 1-5 µM. A microscopy analysis confirmed conidia cell death at 15 µM. This peptide was used in further experiments to assess its efficacy in controlling *B. cinerea* infection on different plant tissues. On bean leaves, this peptide determined a significant reduction of disease symptoms (higher than 95%) at 50 µM, being effective also at 15 µM (75% symptom reduction). Treatment of grapevine leaves and berries at 50 µM showed a significant reduction of disease symptoms of about 70% and 45%, respectively. Several analogs of this peptide, differing in sequence length or C-terminus to decrease synthesis costs, have also been produced and tested. Both changes did not affect peptide efficacy either *in vitro* and against infection on bean leaves. These results show the potential given by small structural modifications of natural secondary metabolites. Ongoing trials are now focusing on the combination of peptides and other natural antimicrobial compounds, with the aim to identify any synergistic effect against *B. cinerea*. Synergy would allow to reduce the fungicide dosage with many benefits, including the reduction of treatment cost, a better toxicity profile and a minimized probability of resistance development.

Using RNA interference to protect crops against fungal pathogens

Mark F Belmonte*, Nicholas Wytinck, Philip L Walker and Steve Whyard

University of Manitoba, Department of Biological Sciences, 50 Sifton Road, Winnipeg, Manitoba, Canada

Sclerotina sclerotiorum, the causal agent of white mold, infects over 450 species of plants worldwide. *Sclerotinia* is a persistent problem for global food production that has traditionally been managed using broad-spectrum fungicides. However, current fungicide strategies have proven less effective and crop rotations fail due to the promiscuous host range of Sclerotinia and the formation of durable resting structures known as sclerotia. Thus, there is an immediate need to manage Sclerotinia using novel species-specific control methods. Our novel strategy exploits the inherent cellular defense process known as RNA interference (RNAi). Upon encountering a double stranded RNA (dsRNA) molecule, the cell processes the dsRNA specifically targeting transcripts with sequence homology. Using a re-designed bioinformatics approach, we identified *Sclerotinia*-specific target genes. RNAi knockdown was confirmed using quantitative real-time PCR on RNA isolated from fungal liquid cultures. dsRNA molecules were screened for growth inhibition on the plant using a system representative of field conditions that showed up to 85% reduction in lesion spread. We then generated transgenic plants over-expressing good quality dsRNA and showed a more profound and prolonged tolerance to the fungus. Finally, I will provide insight into the uptake mechanisms and utility of next generation molecular fungicides and their applicability to control pathogens.

Key words: *Botrytis cinerea*, *Brassica napus*, RNA interference, RNA sequencing, *Sclerotinia sclerotiorum*

Changes along the Mrr1 gene correlate with reduced fludioxonil sensitivity in *Botrytis cinerea*

Charleen Copier[1], Herman Silva[2], Claudio Osorio-Navarro [1,3], Jonathan Maldonado[2,4], Jaime Auger[1] and Marcela Esterio[1]

[1]*Laboratorio de Fitopatología Frutal y Molecular, Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago-Chile.*

[2]*Laboratorio de Genómica Funcional y Bioinformática, Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago-Chile.*

[3]*Centre of Molecular Biology in Plants, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago-Chile.*

[4]*Departamento de Biología, Facultad de Química y Biología, Universidad de Santiago de Chile, Santiago-Chile.*

Gray mold, caused by *Botrytis cinerea,* is the main phytosanitary problem affecting table grape production in Chile. Although different cultural practices are included in the Botrytis management, the greatest efficacy is through fungicides. However, the emergence of fungicide resistance is a central problem in Botrytis control. In contrast to other fungicides, reports showing loss of sensitivity to the phenylpyrrole fludioxonil are scarce worldwide. The fludioxonil molecular target remains elusive, although regulation of cellular secretion mediated by plasma membrane transporters is a potential target. Overexpression of ATP-binding cassette transporter *AtrB* and mutations in the transcription factor Mrr1, involved in the *AtrB* regulation, have been shown in isolates with reduced fludioxonil sensitivity. During the 2018 Chilean table grapes season, 22 from 2400 recovered isolates (0,92%) exceeded the discriminatory dose of 1µg/mL to fludioxonil (EC_{50}). In this study, we investigated the molecular mechanism associated with the loss of sensitivity to this fungicide. Ten sensitive isolates to fludioxonil (Flu^{Sen}) and twenty-two with loss of sensitivity (Flu^{Res}) were evaluated. Four different *AtrB* genotypes were identified with different arrangements in the promoter and the coding region. Mutations N715H, Y737H, A763S, S775A, and E797G in AtrB conditioned a conformational change in the ATP binding site of the coding protein, although without impacting ATP binding, as was revealed by structural modeling. In addition, a direct correlation between the *AtrB* genotype and Flu^{Sen} isolates was not observed. By contrast, two *Mrr1* genotypes were identified, differentiating Flu^{Sen} from Flu^{Res} isolates. In the Flu^{Res} Mrr1 we identified 30 amino acid changes respect to Flu^{Sen} protein. In addition, a highly variable *Mrr1* promoter region was observed in the Flu^{Res} isolates. These data reinforce the role of Mrr1 in decreasing sensitivity to fludioxonil. Remarkably, the infection capacity of Flu^{Sen} and Flu^{Res} were similar in cucumber leaves and detached grape berries, suggesting no fitness cost triggered by fungicide resistant acquisition, in contrast to previous reports in Flu^{Res} isolates worldwide. Currently, formulations combining fludioxonil with other fungicides are available in the market. This situation increases the selection pressure to this molecule and, therefore, the risk of fludioxonil resistant population in table grapes orchards in Chile.

The role of core clock components in secondary metabolism, development, and mycoparasitic interactions between *Trichoderma atroviride* and *Botrytis cinerea*

Marlene Henríquez-Urrutia[1,2], Aldo Seguel-Avello[1,2], Consuelo Olivares-Yánez [1,5], Héctor Guillén-Alonso[3], Robert Winkler[3], Alfredo Herrera-Estrella[4], Paulo Canessa[1,5] and Luis F. Larrondo[1,2,#]

[1] ANID – Millennium Science Initiative Program - Millennium Institute for Integrative Biology (iBio)

[2] Pontificia Universidad Católica de Chile, Biological Sciences Faculty, Molecular Genetics and Microbiology department. Santiago, Chile.

[3] Department of Biotechnology and Biochemistry, Cinvestav Unidad Irapuato, 36824 Irapuato, Mexico

[4] Laboratorio de expresión génica y desarrollo en hongos, Unidad de Genómica Avanzada-LANGEBIO, Cinvestav, 36824 Irapuato, Mexico.

[5] Centro de Biotecnología Vegetal, Facultad de Ciencias de la Vida, Universidad Andrés Bello.

Circadian clocks are essential for individuals' fitness, and recent studies underline their role in the outcome of biological interactions. However, the relevance of circadian clocks in fungal-fungal interactions remains largely unexplored.

Objective: We sought to characterize a functional clock in the biocontrol agent *Trichoderma atroviride* and assess the importance of clock components of both fungi, as well as day/night conditions in the mycoparasitic interaction of this biocontroller against *Botrytis cinerea*.

Methodology and main conclusions: By utilizing luciferase reporters to monitor the *T. atroviride* core-clock, we confirmed the existence of circadian oscillations that are temperature compensated and modulated by environmental cues such as light and temperature. Confrontation assays between WT and clock mutant strains of *T. atroviride* and *B. cinerea*, in constant light or darkness, revealed an inhibitory effect of light on *Trichoderma's* mycoparasitic capabilities. Interestingly, when confrontation assays were performed under light/dark cycles, *Trichoderma's* overgrowth capacity was enhanced when inoculations were at dawn compared to dusk. Deleting the core-clock negative element FRQ in Botrytis, but not in Trichoderma, was vital for the daily differential phenotype, suggesting that the Botrytis clock has a more significant influence in this interaction.

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SESSION V: DISEASE MANAGEMENT II

Chairs: Eduardo Mizubuti & Anne-Sophie Walker

KEYNOTE LECTURE

Botrytis and *Sclerotinia* infections in food industry: risks and management

Gianfranco Romanazzi

Department of Agricultural, Food and Environmental Sciences, Marche Polytechnic University, I-60131, Ancona, Italy

Fungi of the genera *Botrytis* and *Sclerotinia* are able to induce severe loss of production in the field and after harvest on a long list of species of fruit and vegetables. Moreover, they can also affect seed productions with infection and/or contamination of seed lots. Several industries are severily affected by fungi belonging to these two genera, for the effects on the production and following effects on food loss and waste, overall management and follow up to the consumers. Representative species of the two genera are *Botrytis cinerea* and *Sclerotinia sclerotiorum*. While the latter is mainly a field pathogen, with random cases of postharvest contamination (mostly on seeds, where sclerotia can be mixed with the seed lots), *B. cinerea* is among the most important field pathogens and at the same time the ones that induce to highest damage in postharvest stage on a long list of crops, reducing shelf life and producing waste at consumers home. Several industries deals with both pathogens, starting from the industry that produces and places on the market plant protection products, to the industry of the crop production and then the ons involved in postharvest stage (packinghouses, transporters, retailers), and in some cases the requests may be contradictory. The industries of plant protection products and of the crop production need an appropriate management of fungicide applications to reduce the disease in the field, the risk of postharvest infections and the risk of appearing of resistant isolates, mostly for *B. cinerea*, while the retailers are tending to introduce protocols for growers that requires residues of a limited number and concentration of active ingredients, that can result in repeated application of the same fungicide, increasing the risk of appearing of resistant isolates. Innovative strategies for management of diseases caused by fungi of the genera *Botrytis* and *Sclerotinia* that include together with the use of synthetic fungicides the application of biocontrol agents, basic substances and other innovative tools (DSS, prediction models) for can take benefit to all industries in the chain from growers (farm) to the consumer (fork), in a increasing sustainability that takes in consideration the needs of all involved actors.

Exploring *Sclerotinia sclerotiorum* hypovirulence-associated DNA virus 1-infected strain as a plant vaccine to control rapeseed stem rot

Daohong Jiang[1,2], Jiatao Xie[1,2], Jiasen Cheng[1,2] and Yanping Fu[2]

1 State Key Laboratory of Agricultural Microbiology, Huazhong Agricultural University, Hubei Province, Wuhan, China; 2 Hubei Key Laboratory of Plant Pathology, Huazhong Agricultural University, Hubei Province, Wuhan, China.

Sclerotinia sclerotiorum attacks more than 700 plant species, including numerous economically important crops. Rapeseed (*Brassica napus*) stem rot caused by *S. sclerotiorum* is the most important disease on rapeseed in China and it causes about 8.4 billion losses on rapeseed each year under the current control level. Highly efficient and environment-friendly techniques are urgently needed to control rapeseed stem rot. Previously, we isolated and identified a DNA mycovirus, Sclerotinia sclerotiorum hypovirulence-associated DNA virus (SsHADV-1), from a hypovirulent strain DT-8 of *S. sclerotiorum* (Yu et al. 2010, PNAS 107:8387-8392). SsHADV-1 has strong infectivity, its particles could infect *S. sclerotiorum* hyphae directly and could transmit among vegetative incompatible strains of *S. sclerotiorum* efficiently; when inoculated on plants, the virus-infected strain could not induce typical lesion. SsAHDV-1 and mycophagous insect (*Lycoriella ingenua*) underwent mutualistically symbiosis and the virus drives the insect as transmission vector (Liu et al. 2016, PNAS 113: 12803-12808). These traits suggest that SsHADV-1 has great potential for biological control of rapeseed stem rot. When we delivered the hyphal fragments of virus-infected strain on rapeseed, the virus and its infected-strain could reduce the disease index of stem rot up to 70% and significantly enhance seed yield. More importantly, the virus-infected strain could live on rapeseed though its virulence was fully suppressed by SsHADV-1. We labelled the virus-infected strain with mCherry gene to understand whether the virus-infected strain grows on the rapeseed surface or in plant tissues, and found that the virus-infected strain grew in both intercellular space and plant cells. The virus-infected strain when growing on plants could promote plant growth and enhance plant resistance against *S. sclerotiorum* by regulating the defence, hormone signalling and circadian rhythm pathways of rapeseed. Thus, SsHADV-1 converts its host from a necrotrophic pathogen to a reciprocal endophytic fungus, and based on this finding, we developed a novel strategy for controlling rapeseed stem rot using the virus-infected strain as "plant vaccine" to improve rapeseed health and released SsHADV-1 to rapeseed field (Zhang et al. 2020, Mol Plant 13: 1420-1433). The "plant vaccine" has exhibited high efficacy in the field when it was used to treat rapeseed seeds.

Understanding the interaction between calcium, cut roses, and *Botrytis cinerea.*

Melissa Muñoz, James E. Faust, Guido Schnabel and Chistopher Saski.

Plant and Environmental Sciences Department, Clemson University, Clemson, South Carolina, USA.

Botrytis cinerea causes Botrytis blight in cut flower roses. Fungicide applications are frequently used as pre-and post-harvest treatments to reduce disease incidence and severity, which is a concern for human and environmental safety and has led to the development of fungicide-resistant isolates. Calcium (Ca) applications have shown great potential as an alternative strategy for Botrytis blight in ornamentals. The objectives of this research were: to evaluate the effect of post-harvest Ca dips on Botrytis blight severity on cut roses, to determine the efficacy of post-harvest Ca dips to increase Ca concentration in the petal tissue, and to evaluate the potential metabolic pathways involved in the interaction between cut roses, Ca, and *B. cinerea.* To evaluate the first two objectives, 15 sec. post-harvest dips of whole flower heads were performed in two commercial greenhouses in Colombia, South America, using calcium chloride ($CaCl_2$) on the Botrytis-sensitive cultivar Orange Crush at rates of 0 and 2000 ppm Ca while a hydrogen peroxide treatment was used as a commercial control. The flowers were then shipped to Clemson University for evaluation. Upon arrival, eight flowers per treatment were placed in a humid chamber for disease severity evaluation, while another eight flowers were used for tissue analysis. For the third objective, harvested 'Orange Crush' roses were treated with postharvest dips of either 0 or 2000 ppm Ca. 24h after the dips, six roses per treatment were inoculated by spray with a *B. cinerea* spore suspension at 1×10^5 spores/ml, while another six roses per treatment were sprayed with sterile deionized water as a control. The flowers were kept for 4 days in a humid chamber to allow symptom development. Then, 200 mg of petal tissue were harvested, ground, and used for global metabolomics evaluation using the UHPLC-Obitrap fusion method. Our results show that postharvest Ca dips were effective at increasing Ca concentration in petal tissue in the epidermis and mesophile, and also reduced Botrytis blight severity. Additionally, gene set enrichment analysis showed that during the Ca/*B. cinerea* interaction, the cysteine, methionine, phenylalanine, purine, fructose, and mannose metabolism, and glucosinolate biosynthesis pathways were upregulated by the application of Ca treatment, while the steroid biosynthesis and alpha-linolenic acid metabolism pathways were downregulated. Mannose metabolism and glucosinolate biosynthesis were significantly upregulated in the inoculated control (0 ppm Ca) treatment, suggesting possible signaling and regulation mediated by Ca applications.

A novel ARMS-PCR for the detection of *Bcpos5* mutations conferring resistance to AP fungicides in *Botrytis cinerea* and use of CRISPR/Cas9 editing for characterization of the resistant mutants

Georgios Sofianos, Athanasios Petmezas, Vasilios Kosidis, Anastasios Samaras and Georgios Karaoglanidis

Aristotle University of Thessaloniki, School of Agriculture, Department of Plant Pathology, Thessaloniki, Greece

Despite the long-standing presence of anilinopyrimidine-resistant strains in *Botrytis cinerea* populations throughout the world, the precise resistance mechanism has only recently been elucidated. The molecular target of APs is of mitochondrial origin and the *Bcpos5* gene mutations G408V, L412V and L412F are associated with resistance to APs. The diversity of mutations necessitates the availability of a tool for their rapid identification. Therefore, an adapted Tetra-primer ARMS PCR was developed in this study. The PCR yielded a 702bp band for all isolates while, the presence of a 470bp or 252bp band corresponded to F and V substitutions, respectively. To confirm the presence of the third mutation (G408V), the PCR products with a single band (702bp) were further digested by enzyme MlyI yielding 467bp and 235bp bands in the G408V mutants. The results were verified by Sanger sequencing. The Tetra-primer ARMS PCR method was validated in a set of 82 pathogen isolates collected from strawberry and tomato fields and revealed that L412F, L412V and G408V were occurring at frequencies of 69%, 7% and 1.5%, respectively. The results of the study suggest that this ARMS-PCR technique can be a useful tool for sensate, accurate and rapid identification of *Bcpos5* mutations in *B. cinerea*.

To further characterize APs-resistance mutations, B.05 reference strain was transformed with L412F and L412V using the CRISPR/Cas9 technique and homologous recombination. To determine any potential fitness cost associated with the aforementioned mutations, a set of isolates possessing these mutations (both field and transformed ones) were subjected to evaluation of certain fitness parameters and determination of L412F and L412V effects on the sensitivity to cyprodinil of the transformed strains.

In conclusion, it is obvious that CRISPR/Cas9 editing is of vital role regarding resistance studies, allowing the precise testing of single mutations and their effects, unaffected by other factors, and that the results of fitness assessment indicate the ease with which AP-resistant isolates can spread and settle in the field.

Novel SDHI molecules may change *sdh*B mutation frequencies and select for new mutations in *Botrytis cinerea* populations– nematicidal applications of fluopyram as a case study

Anastasios Samaras[1], Chloe Chatzipetrou[1], Juergen Derpman[2], Alexios Kalpakidis[1] and George Karaoglanidis[1]

[1]Plant Pathology Laboratory, Department of Agriculture, Aristotle University of Thessaloniki, Thessaloniki, Greece. [2]Bayer AG, Division CropScience, Research, Disease Control, Monheim, Germany.

Succinate dehydrogenase inhibitors (SDHIs) constitute a major fungicide group used against *Botrytis cinerea*. Boscalid was the first SDHI used against gray mold but it selected very fast for resistance associated with several *sdh* mutations. Recently, other molecules such as fluopyram, isopyrazam etc. were registered for use on several crops. In addition, in greenhouse grown vegetables fluopyram is used as a nematicide applied in soil. This study was conducted to test the hypothesis that nematicidal applications of fluopyram impose a risk for resistance selection in *B. cinerea*.

Extensive samplings were conducted during 2018 (n=995) and 2019 (n=403), in tomato greenhouses located in 2 regions of Greece that had been divided in groups of greenhouses that received fluopyram applications as nematicide and greenhouses without any fluopyram application. A predominance of isolates with double resistance to both boscalid and fluopyram (B^RF^R) was found in fluopyram-treated greenhouses, while in fluopyram-untreated greenhouses the sensitive isolates were dominant, followed by isolates that were singly resistant to boscalid (B^R). Interestingly, in fluopyram–treated greenhouses were detected isolates of the B^RF^R phenotype or isolates with slightly reduced sensitivity to fluopyram but not to boscalid. Identification of mutations associated with these phenotypes revealed that N230I was the predominant mutation of the B^RF^R isolates. Isolates with slightly reduced sensitivity levels to fluopyram were possessing the I274D in *sdh*B, G37S in *sdh*C or the V9A and S88F in *sdh*D and are reported for 1st time in fungal field populations. Information on the cross-resistance relationships among SDHIs and the fitness of these mutants will be provided. In addition, to investigate the effect of V9A *(sdh*D) and G37 (*sdh*C), we proceeded to transformation of the sensitive reference strain B0.5, with the method of homologous replacement with a coding sequencing of *sdh* gene from resistant isolates with only one SNP. Phenotypic characterization of transformed strains confirmed the correlation between those mutations and sensitivity to fluopyram.

In conclusion, this study suggests that fluopyram treatments as nematicide may alter *B. cinerea* resistance frequencies to SDHIs and the respective frequencies of *sdh* mutations with implications to gray mold management. The new *sdh* have to further been characterized to determine the risk for further selection in crops heavily treated with botryticides.

Is the protective efficacy of microbial biocontrol agents stable regardless of the diversity of *Sclerotinia sclerotiorum* strains?

Estelle Turc[1,2], Noadya Monnier[1], Philippe Nicot[2] and Marc Bardin[2].

[1]*Groupe Eléphant Vert France, Serris, France.* [2]*INRAE, Pathologie Végétale, Montfavet, France*

Sclerotinia sclerotiorum has a destructive impact on oilseed rape crop and its control still relies mainly on chemicals. Therefore, the development of biocontrol agents is of great interest to reduce the amount of chemical inputs in this disease management.

Two candidate microorganisms among several others isolated in our laboratory gave promising results under controlled conditions and in experimental fields to protect oilseed rape against *S. sclerotiorum*. Preliminary results concerning the mode of action of these microorganisms have shown a reduction in the *in vitro* development of *S. sclerotiorum* when confronted with biocontrol microorganisms, presumably due to the secretion of diffusible antifungal molecules.

Different biotic and abiotic factors can have an impact on the level of protection provided by a microbial biocontrol agent. Among these factors, the reduced sensitivity of certain strains of the pathogen to the biocontrol agent may affect their protective efficacy in the field. Thus, the aim of this study was to determine whether the effect of the biocontrol microorganisms is stable against different strains of *S. sclerotiorum*.

To this end, the inhibition of the development of the fungal pathogen by the two candidate microorganisms was evaluated on dual culture plate assays against fifty strains of *S. sclerotiorum*. These *in vitro* assays revealed a significant effect of *S. sclerotiorum* strains on the antifungal activity of both biocontrol agents, suggesting that some strains of the pathogen are significantly less impeded in their development by the biocontrol agents than others. These results also allowed the selection of strains that represent the diversity of sensitivity to the biocontrol microorganisms for testing on plant detached leaves of oilseed rape. These results will be further discussed.

SESSION V: DISEASE MANAGEMENT II

POSTERS

Potential fungicide activity of endophytic fungi isolated from ginger against *Botrytis cinerea*

Rogelio Borrego[1], Alejandro Bódalo[1], Victoria-E. González-Rodríguez[1], Carlos Garrido[1], María Carbú[1], María Dolores Vela-Delgado[2], Hernando José Bolívar Anillo[3], M. Teresa Gutierrez[1] and Jesús M. Cantoral[1]

[1]Departamento de Biomedicina, Biotecnología y Salud Pública, Laboratorio de Microbiología, Facultad de Ciencias del Mar y Ambientales, Universidad de Cádiz, 11510, Puerto Real, Spain.
[2]IFAPA Rancho de la Merced, Sede CHIPIONA. Camino Esparragosa s/n. 11.550 Chipiona, Spain.
[3]Programa de Microbiología, Facultad de Ciencias Básicas y Biomédicas, Universidad Simón Bolívar, Barranquilla, Colombia.

Grey mould is a plant disease caused by phytopathogenic fungi *Botrytis cinerea*, which infects a wide variety of plants and causes important agricultural damages. It is well known the capacity of some fungi (e.g. genus *Trichoderma*) of killing another fungi, making possible its use as biocontrol agents.

Endophytic fungi are those that live inside a host plant without injuring it but, on the contrary, establishing a symbiotic relationship with, and both organisms obtain benefits. Endophytic fungi have been widely studied due to its high potential in biotechnology as, for example, biocontrol agents. Ginger (*Zingiber officinale*) is a medicinal plant most used traditionally in Asia, and it hosts a wide variety of endophytic fungi very interesting in biotechnology. Therefore, the aim of this study is to find endophytic fungi from ginger with capacity to stop the growth of *Botrytis cinerea* and, consequently, try to solve the problem of grey mould disease.

We isolated nine different species of fungi from ginger tubers by classic microbiology techniques. They were identified applying PCR techniques using three different genes. The fungi are facing individually in Petri dishes against phytopathogenic fungi *Botrytis cinerea* in order to evaluate their fungicide activity. Furthermore, we have made some fermentations of the fungi in different times, extracts obtained will be concentrated and also faced against *Botrytis cinerea* to detect if they have antifungal properties.

Our results show interesting antifungical control of the fungi and/or their extracts to be able to stop the growth of *Botrytis cinerea.* They could be an alternative for discovering new strategies of control for grey mould, avoiding the use of damaging methods as chemical fungicides.

Adepidyn™ baseline sensitivity against *Botrytis cinerea* isolates recovered from table grapes and wine grapevines of the main Chilean producing regions

Marcela Esterio[1], Mauricio Rubilar[1], Charleen Copier [1], Claudio Osorio-Navarro[1,2], Santiago Valdes[3], Paulo Rivara[3], Gabriel Scalliet[4] and Jaime Auger[1]

[1]Laboratorio de Fitopatología Frutal y Molecular, Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago-Chile.

[2]Centre of Molecular Biology in Plants, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago-Chile.

[3] Syngenta S.A., Chile.

[4]Syngenta, Switzerland.

The gray mold (Botrytis cinerea (Bc)) is the main phytopathological problem of grapevines, and the chemical control is fundamental. Actually, the new succinate dehydrogenase inhibitors (SDHI) have an important role on *Bc* bloom control. The objective of this study was to determine the sensitivity behaviour of *Bc* isolates that had never before being subjected to adepidyn™. This carboxamide ready to be register in Chile for the botrytis grapevines control. For this purpose, during the 2016/17 season, the average, min, max and median EC_{50} values for conidial germination (CG) and germination tube elongation (GTE) were determined on 200 *Bc* isolates recovered in bloom from 40 fields located in the main grape producing areas in Chile (104 and 96 *Bc* isolates recovered from table grapes and wine grapes cvs., respectively). The 200 isolates were subjected to increasing concentrations of adepidyn™ amended in Water-Agar medium (0; 0.00001; 0.0001; 0.001; 0.01; 0.1; 1 and 10 µg/mL), being evaluated by CG above 100 conidia and GTE above 10 conidia per each concentration. The same *Bc* isolates were subjected to increasing concentrations of fludioxonil, being determined the EC_{50} value according to the mycelial growth (MG), in amended Sisler medium with 0; 0.025; 0.05; 0.1; 0.5; 1 and 10 µg/mL. In both cases, 3 replications were considered for each concentration. The min, max, median and average EC_{50} values obtained for adepidyn™ considering the 200 isolates for CG were 0.003; 1,306; 0.023 and 0.054µg/mL and for GTE were 0.00003; 0.00229, 0.00027 and 0.00038 µg/mL, respectively. In table grapes, EC_{50} values were always higher than the wine grapes *Bc* isolates. The adepidyn EC_{50} value of the 68% of the isolates were between 0.01 µg/mL and 0.1 µg/mL; and the 99.50% of the isolates were less than 1 µg/mL and only one exceeded this range (1,306 µg/mL). The MG, EC_{50} average value, for fludioxonil were 0.046 µg/mL and 0.025 µg/mL for table grapes and wine cultivars, respectively. The atypical *Bc* isolate with a high EC_{50} value for adepidyn™, presented a low EC_{50} for fludioxonil (0.037 µg/mL). These results pointed out that adepidyn™ and its blending (adepidyn™&fludioxonil) is an interesting and effective alternative to be used in the management of botrytis in table and wine grapes; and the EC_{50} values obtained will be the reference point for the future monitoring of adepidyn™ sensitivity in grapevines in Chile.

Biocontrol of *Botrytis cinerea* as influenced by environmental conditions

Giorgia Fedele[1], Elisa González-Domínguez[2] and Vittorio Rossi[1]

[1] DiProVeS, Università Cattolica del Sacro Cuore, Piacenza, Italy. [2]Horta srl, Piacenza, Italy.

In recent years, researchers have been increasingly explored alternatives to chemical pesticides for the control of Botrytis bunch rot (BBR) on grapevines, caused by *Botrytis cinerea*, including the use of biological control agents (BCAs). Some commercial BCAs are available for the control of BBR but their practical use is still limited. One likely reason is the inconsistent efficacy of BCAs under field conditions, which may be related to the complex interactions among the target pathogen, the host plant, and the BCA population in a changing environment. So that, the colonization rate and the efficacy of the BCA, as well as the pathogen growth and infection, are all influenced by environmental conditions, such as temperature (T) and relative humidity (RH). To achieve an effective integration of BCAs in a disease management program is then relevant to know: i) the life cycle of both, the pathogen and the BCA; ii) the mode of action of the BCA against the target pathogen; and iii) how the two are influenced by the environment.

Recently, a generic mathematical model for the biocontrol of diseases was developed and applied to grapevine-*Botrytis* pathosystem and BCAs for BBR control. The variance in simulated biocontrol efficacy highlighted that the most important factors affecting BCA efficacy were those related to environmental requirements for BCA growth and survival.

In the present work, commercial BCAs for the biocontrol of BBR were applied to different substrates (i.e., bunch trash and artificial substrates simulating the chemical composition of grape berries at different growth stages) and were then incubated at different conditions of T and RH. After 1 to 13 days of incubation (BCA colonization period), the number of colony forming units (CFUs) was assessed. For each substrate, equations were developed that accounted for the combined effects of the environmental conditions and BCA colonization period. After field validation, the equations would help farmers in BCA selection for any application based on grape growth stages and weather conditions at the time of treatment and in the following days. The equations would also help in predicting how long a first BCA application may remain effective and thereby help farmers decide whether and when a second BCA application may be needed.

Fungal species screening from *Vitis vinifera* native microbiome determines the *Botrytis* control ability of *Trichoderma gambsii* strains

Gonzalo Gutiérrez[1], Claudio Osorio-Navarro [1,2], Charleen Copier[1], Felipe Durán[1], Madelaine Azocar[1], Pablo Kauer[3], Eduardo Donoso[4], Martín Merino[5], Paulo Rivara[6], Juan Carlos Sepúlveda[7], Herman Silva[8], Jaime Auger [1] and Marcela Esterio[1]

[1]Laboratorio de Fitopatología Frutal y Molecular, Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago-Chile. [2]Centre of Molecular Biology in Plants, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago-Chile. [3]Basf Chile S.A., Santiago-Chile. [4]Bio Insumos Nativa, Santiago-Chile. [5]Summit Agro Chile SpA., Santiago-Chile. [6]Syngenta S.A., Santiago-Chile. [7]FEDEFRUTA, Santiago-Chile. [8]Laboratorio de Genómica funcional y Bioinformática, Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago-Chile.

Botrytis cinerea rots represent enormous losses during table grape production. Although chemical control is the primary tool for *B. cinerea* management, significant efforts have been made to find ecologically sustainable options. Biological control is a powerful alternative to fungicide use, mainly due to the rapid colonization capacity offered by microorganisms, counteracting the advance of the pathogen. Different species of bacteria and fungi of *Bacillus* and *Trichoderma* genus are used as Biological Control Agents (BCA) in plant species of commercial interest. The native microbiota is specifically adapted to conditions of ecological niche within the plant tissue, including the fluctuations caused by plant physiological changes. For this reason, the native microbiota has been the focus of the new BCA investigations. In this work, the native fungal microbiota was isolated from flowers and berries of *Vitis vinifera* cv. Thompson Seedless and used to evaluate its potential as BCA against *B. cinerea*. All morphologically different isolates (n = 85) obtained from 2400 flowers and berries were collected from four fields in two regions of Chilean Central Valley. The isolates were identified by multilocus molecular phylogeny at the species level. Twenty genera were detected, where *Botrytis* spp. and *Rhizopus* spp. were the most abundant, followed by *Alternaria* spp. (22%), *Penicillium* spp. (18%), *Trichoderma* spp. (12%) and *Epicoccum* spp. (9%). Notably, basidiomycetes of the genus *Coprinellus* and *Bjerkandera* were detected for the first time associated with *V. vinifera*. The composition of the fungal microbiota was different between fields and this was persistent from flowers to berries. Next, isolates associated with grapevine diseases were discarded, and dual cultures were developed confronting isolates from selected species with fungicide-resistant *B. cinerea*. The *Trichoderma* genus was the most diverse and the best performing in the biocontrol tests. Three isolates from *Trichoderma gambsii* obtained a percentage of radial growth inhibition >50%. One of them was not affected by fungicides tested, suggesting their potential use in combination with commercial products. Field validation of the selected strains will offer an improved alternative for *Botrytis* control. Finally, the native grapevine microbiota database generated in this work allowed the design of a specific new *B. cinerea* diagnostic strategy from plant tissue by qPCR-HRM. Founding: Proyecto FIA PYT-2020-0208.

The potencial of the RNAi technology, via SIGS, in the control of *Botrytis cinerea* in horticultural crops

Alba López-Laguna[1,2], Alejandra Vielba-Fernández[1,2], Alejandro Pérez-García[1,2] and Dolores Fernández-Ortuño[1,2]

[1]*Dpto. de Microbiología, Facultad de Ciencias, Universidad de Málaga, Málaga, Spain;*

[2]*Dpto. de Microbiología y Protección de Cultivos, Instituto de Hortofruticultura Subtropical y Mediterránea (IHSM-UMA-CSIC) "La Mayora", Campus de Teatinos, Málaga, Spain*

Botrytis cinerea is undoubtedly one of the most important limiting factors for crop production worldwide, as it demonstrated by the enormous annual intake of fungicides used for their control to avoid crop losses that can reach 40% (Petrasch et al. 2019, *Mol. Plant Pathol*, 20, 877–892). However, this fungus has been categorized by FRAC as a high-risk pathogen for fungicide resistance development. Another problem is related with the diversity of fungicides available to growers, which according with the current European legislation on pesticides and the European Green Deal, will be reduced by 50% by 2030. For all this, new low-impact sustainable solutions, obtained through new phytoprotection tools, to control *B. cinerea* are needed. In this study, we intend to check if some emerging strategies such as RNA interference technology (RNAi) could be valid sustainable solution and alternative to the use of conventional chemical fungicides for the control of *B. cinerea* in crops of relevance. To achieve this goal, the SIGS (spray-induced gene silencing) approach, which concerns the exogenous application of double-stranded RNA (dsRNA), was tested. For it, ten double-stranded RNA (dsRNAs) were designed against the fungicide target's genes [*tub2* (β-tubulin), *bos1* (histidine kinase class III), *cyp51* (C14-demethylase in sterol biosynthesis), *cytb* (cytochrome b), *erg27* (3-ketoreductase), *mrr1* (transcription factor Mrr1) and *sdhB* (subunit B of the succinate-dehydrogenase)] and genes encoding proteins involved in virulence/pathogenicity of this fungus (Choquer et al. 2007, FEMS Microbiol. Lett., 277, 1–10) such as *sod1* (superoxide dismutase), *bmp1* (MAPK kinase) and *Bcpg2* (endopolygalacturonase). The preliminary results obtained in *in vitro* tests have shown that the application of the different dsRNAs, individually and in combination, have significantly reduced the development of the fungus on different culture media. In addition, this reduction was very promising on detached fruit and *in planta* assays, demonstrating the potential of this technique in the control of *B. cinerea*. On the other hand, the sustained release of the dsRNA-fungicides using nanoparticles as a carrier or stabilizer has also been analyzed. Today, more than ever, we need have new molecules with fungicidal action, such as the RNAi-based fungicides, for their inclusion in the rotations of the different *Botrytis*-control programs in the field.

Patterns of fungicide resistance and genetic diversity in *Botrytis cinerea*: implications for disease management in Lombardy

Demetrio Marcianò [1], Giuliana Maddalena [1], Federico Massi [1] and Silvia Laura Toffolatti [1]

[1] *Università degli Studi di Milano, Dipartimento di Scienze Agrarie e Ambientali (DISAA), via Celoria 2, 20133, Milano, Italy.*

Grey mould infections, caused by *Botrytis cinerea,* regularly occur in Lombard vineyards (northern Italy), with variable levels. Disease management relies on the application of 1-4 treatments with fungicides possessing a single-site mode of action (succinate dehydrogenase inhibitors - SDHIs; anilinopirimidines - APs; phenylpyrroles - PPs; and ketoreductase inhibitors - KRIs). Despite the negative impact of the pathogen on grape production, little information was available on the composition of *B. cinerea* population in the region. In this study the fungicide resistance and molecular profiles of *B. cinerea* strains isolated from four viticultural areas located in Lombardy were determined to get useful information for the management of the disease.

Seven-hundred and twenty *B. cinerea* strains, isolated from 36 vineyards, were characterized for sensitivity to boscalid (SDHI), cyprodinil (AP), fludioxonil (PP) and fenhexamid (KRI). Moreover, 317 isolates were completely genotyped for six microsatellite *loci* and characterized for the presence of transposable elements (*Boty* and *Flipper*) and for the mating type.

Field populations of *B. cinerea* showed a relatively low frequency of strains resistant to a single (0.1-5 % of the strains) or two fungicide classes (0.8 % of the strains), uniformly distributed over the four provinces, except for the fludioxonil–resistant strains which mainly concentrated in a single province (Sondrio). Resistance to members of SDHI and KRI associated with known mutations at the *sdh*B (H272Y/R) and *erg27* (P238S or I232M) genes and with two potentially new mutations in *sdhC* (A187F) and in *sdhD* (I189L) for SDHI. No fitness costs were found in resistant strains compared to the sensitive ones.

B. cinerea populations showed a high genotypic diversity, different frequencies of transposable elements and a mixed mode of reproduction (sexual and asexual), indicating a high evolutionary potential and adaptive capacity. Even if, at regional level, *B. cinerea* strains seemed to belong to a large and interconnected population, the Sondrio subpopulation showed a small but statistically significant genetic differentiation from the others.

Taken together, these results suggest that grey mould management should be implemented, at local level, taking into consideration the peculiar situation of the Sondrio province, and at regional level, to avoid the selection and spread of resistant strains among provinces.

Investigating the cellular response to α-tomatine-induced plasma membrane damage in *Botrytis cinerea*

Linda Matz, Anne Oostlander, Ulrike Brandt and André Fleißner

Institute of Genetics, Technische Universität Braunschweig, Spielmannstraße 7, 38106 Braunschweig, Germany

Botrytis cinerea is a ubiquitous plant pathogenic fungus that causes large annual yield losses. Host plants include many important crops such as strawberry and tomato. As part of their defense against fungal pathogens, tomato plants produce the saponin α-tomatine, which is capable of forming specific complexes with ergosterol, the main sterol of the fungal plasma membrane. The complex formation results in the formation of pores which cause loss of membrane integrity and subsequent cell death. Pathogenic fungi such as *B. cinerea* possess resistance mechanisms which nevertheless allow infection of the plant, such as degradation of α-tomatine to the less harmful β-tomatine and D-xylose. Degradation, however, takes some time and the cell needs more rapid mechanism to respond to occurring membrane damage. Recently, novel membrane repair mechanisms have been identified in *Neurospora crassa*, which involve the calcium binding penta-EF-hand protein PEF1, and annexins ANX14 and ANXC4. Deletion of these genes in *N. crassa* lead to a strongly increased sensitivity to membrane-disruptive drugs, such as tomatine, and fusion-induced injuries, suggesting that membrane repair is part of the cellular response against membrane disruption.

In this study, we identified the PEF1 homologous protein of *B. cinerea* and characterized its cellular function during membrane stress and pathogenic growth. In spore germlings, BcPEF1-GFP is rapidly recruited to damaged membrane areas after the treatment with α-tomatine. Stress tolerance assays show, however, that in contrast to *N. crassa*, the deletion mutant is not more sensitive to α-tomatine. In infection assays, no difference was observed between the deletion mutants and the wild type. These results suggest that BcPEF1 is involved in the cellular response to membrane damage, but that *B. cinerea* possesses additional efficient ways of tolerating α-tomatine. We are currently working on characterizing the annexins of *B. cinerea* and identifying their functions in membrane repair. It will be of great interest, to determine the specific contributions of the different resistance mechanisms, including membrane repair, drug efflux and drug degradation, in future studies.

Shining a light on sustainability:

The effects of far-red: blue light ratios on the development of *Botrytis cinerea*

Joshua Molligan and Valérie Gravel

Department of Plant Science, McGill University, Sainte-Anne-de-Bellevue, Québec, Canada

In Quebec the production of greenhouse cultivated strawberries (*Fragaria* x *ananassa* Duch.) has been steadily increasing to meet the year-round demand of consumers. Indoor production settings can provide a suitable environment for the growth and development of fungal pathogens such as *Botrytis cinerea* Pers. To manage disease within a greenhouse, growers often rely on an integrated approach of environmental and cultural controls as well as periodic pesticide applications. Although, the over-application of fungicides in conjunction with the genetic plasticity of this pathogen can result in the occurrence of multifungicidal resistant isolates. Therefore, it is necessary to reexamine environmental factors that contribute to the viability of this pathogen. The objectives of this study were to test the effects of far-red: blue light ratios (1:1, 1:5, and 5:1) on the radial growth and sporulation of *Botrytis cinerea in-vitro.* The blue light wavelength used in this study was 450 nm and the far-red light wavelength used was 720 nm. LED lights (U Technology Corporation, Calgary, Alberta, Canada) were placed one meter above petri dishes containing a 5mm mycelial disk on PDA media. The timing of light exposure was set to 24 hours, and the controls used for this experiment were fluorescent light and complete darkness. Radial growth rate was measured for the initial seven days, and sporulation was analyzed from the same units after a total of fourteen days. Sporulation inhibition was also measured from samples kept at seven days in fluorescent light then placed under far-red: blue light ratios for an additional seven days. The results of this study showed a reduction in the rate of radial growth for all samples under far-red: blue light ratios with the induction of stationary growth after four days. The most effective treatment was a ratio of 5:1 followed by 1:5, showing a significant reduction in radial growth compared to either control. The sporulation of samples under constant far-red: blue light ratios was considerably reduced compared to the controls of fluorescent light and darkness. The inhibition of sporulation these light ratios have was validated further when additional samples were transferred from fluorescent light to each of the three light ratios. This study has validated further experimentation on plant material *in vivo*.

Real-time design of *Botrytis cinerea* control programs based on plant tissue detection of fenhexamid- and boscalid-resistance genotypes using qPCR-HRM (High Resolution Melting)

Claudio Osorio-Navarro[1,2], Charleen Copier[1], Felipe Durán[1], Madelaine Azocar[1], Gonzalo Gutiérrez[1], Pablo Kauer[3], Eduardo Donoso[4], Martín Merino[5], Paulo Rivara[6], Juan Carlos Sepúlveda[7], Herman Silva[8], Jaime Auger[1] and Marcela Esterio[1]

[1]Laboratorio de Fitopatología Frutal y Molecular, Departamento de Sanidad Vegetal, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago-Chile. [2]Centre of Molecular Biology in Plants, Departamento de Biología, Facultad de Ciencias, Universidad de Chile, Santiago-Chile. [3]Basf Chile S.A., Santiago-Chile. [4]Bio Insumos Nativa, Santiago-Chile. [5]Summit Agro Chile SpA., Santiago-Chile. [6]Syngenta S.A., Santiago-Chile. [7]FEDEFRUTA, Santiago-Chile. [8]Laboratorio de Genómica funcional y Bioinformática, Departamento de Producción Agrícola, Facultad de Ciencias Agronómicas, Universidad de Chile, Santiago-Chile

Botrytis cinerea is one of the main threats to table grape production. Its control depends on cultural practices but fundamentally on synthetic fungicides. However, a prolific and genetically variable offspring of *Botrytis* per season has conditioned the emergence of resistance to the main fungicide families. Mutations in the molecular targets of these fungicides determine their loss of efficacy, in some cases keeping unchanged their ability to compete and virulence. This work generated a rapid *Botrytis* genotyping detection system from grapevine flowers and berries based on qPCR-HRM to design field control programs in real-time. The *Erg27* and *SdhB* genes encoding the molecular targets of hydroxyanilides (fenhexamid) and carboxamides (boscalid), respectively, were chosen as the object of study. Primers flanking the regions of interest within each gene were designed, adjusted for use, and validated with control isolates of *B. cinerea* (*Erg27*$^{Phe412Ser/Val/Iso}$; *SdhB*$^{Pro225His-His272Arg/Tyr}$). The genome of *Vitis vinifera* or that of fungal microorganisms isolated from flowers and berries (N= 50 species) did not interfere with the generated detection system. Distinctive dissociation profiles were obtained for each mutation of interest in Botrytis after plant tissue assays. The technique was successfully evaluated and validated as a diagnostic tool taking flowers and berries collected from fields located in the Central Valley of Chile (2020-2021 season). On average, detection results are obtained 24 hours for every 48 samples processed. The results show a broad coexistence of *Botrytis* genotypes in flowers that decreases in berries, confirming the high susceptibility of flowers to this pathogen. The control programs developed according to the *Botrytis* genotypes detected in the tissue are more effective than those that use traditional microbiological techniques based on Botrytis population sensibility (EC_{50}). The development of this tool confers an auspicious system for the *Botrytis* control in table grapes and other agricultural crops affected by this pathogen. Founding: Proyecto FIA PYT-2020-0208.

Multiple and Multidrug resistance in *Botrytis cinerea*: Molecular mechanisms of MLR/MDR phenotypes in Greece and effects of co-existence of different resistance mechanisms on fungicide sensitivity

Georgios Sofianos, Anastasios Samaras and Georgios Karaoglanidis

Aristotle University of Thessaloniki, School of Agriculture, Department of Plant Pathology, Thessaloniki, Greece

Botrytis cinerea is a high risk pathogen for resistance development. Within the fungal populations, strains have built up that possess multiple mutations in different target genes leading to Multiple Resistance (MLR) or mutations associated with overexpression of efflux transporters leading to Multidrug Resistance (MDR). These types of resistance are a major threat and their successful management is a major challenge. The current study was initiated to: a) determine frequencies of MLR/MDR strains in populations originating from several crops, b) identify the type(s) of MDR that occur in Greece and c) determine interactions between MLR and MDR in the level of sensitivity to botryticides.

The frequencies of MLR/MDR phenotypes, were determined in 515 isolates that were subjected to bioassays using discriminatory concentrations of thiophanate-methyl, iprodione, cyprodinil, fenhexamid, boscalid, fluopyram, fludioxonil, pyraclostrobin and tolnaftate. Interestingly 7,8 and 31,3% of isolates from strawberry and rootstock seedlings were resistant to every single fungicide class, while MDR phenotypes from strawberry, rootstock seedlings and tomato accounted for 26, 87 and 13,4% respectively.

The MLR and MDR isolates were further molecularly analysed regarding genes *erg27*, *sdhB, Bcpos5* and *Mrr1*, each responsible for resistance to fenhexamid, boscalid and fluopyram, cyprodinil and MDR, respectively. The different mutations' presence and frequencies were determined along with a new mutation in *Mrr1* leading to MDR. MDR isolates were characterised as MDR1 or MDR1h based on the presence of a 3-bp deletion in *Mrr1*. MDR1h was predominant in isolates from rootstocks and MDR1 from tomato and strawberry, whereas the most frequent target site mutations were F412S *(erg27)*, H272R *(sdhB)* and L412F *(Bcpos5).*

To determine whether the accumulation of target-site mutations along with MDR mutations exhibits an additive effect concerning fungicide resistance, the sensitivity of isolates possessing the predominant target site mutations was calculated both in the presence and the absence of MDR-associated mutations. EC_{50} in cyprodinil and boscalid increased to about 2-fold in the presence of MDR mutations, while there was no difference for fenhexamid.

In conclusion, MLR/MDR frequencies are notably high in heavily-treated crops in Greece and the combination of MLR and MDR mutations leads to even higher fungicide resistance levels, highlighting the importance of resistance management.