ECFG15

ROME • ITALY

Colletotrichum workshop 2020

15TH EUROPEAN CONFERENCE ON FUNGAL GENETICS

Organizers: Riccardo Baroncelli and Serenella Sukno

MONDAY THE 17TH FEBRUARY 2020 | ROME, ITALY

Program

9:00 - 9:15 Arrival of participants and Welcome

09:15 Michael Thon, University of Salamanca, Spain
Horizontal gene transfer contributes to virulence in *Colletotrichum*

09:40 Pamela Gan, Riken, Japan
Genome rearrangements drive evolution of virulence-related genes in the genomes of *Colletotrichum gloeosporioides* species complex

10:05 Vladimiro Guarnaccia, University of Torino, Italy
***Colletotrichum* species diversity on aromatic and ornamental plant hosts in Italy**

10:45 - 11:15 Coffee Break

11:15 Noam Alkan, the Volcani Center, Israel
Glycosylated flavonoids - fruit hidden arsenal against fungal pathogens

11:40 Lars Voll, Philipps-University Marburg, Germany
Reactive Oxygen Species dosage in Arabidopsis chloroplasts improves resistance towards *Colletotrichum higginsianum* in a WRKY33-dependent fashion

12:05 Bastien Bissaro, Aix-Marseille Université, France
Investigating the role of a fungal oxidase-peroxidase tandem in plant pathogenicity

12:45 - 14:00 Lunch Break

14:00 Carmit Ziv, the Volcani Center, Israel
The effect of fruit sugar level on the pathogenicity mechanism and host response during *Colletotrichum* infection of red tomatoes

14:25 Gaetan Le Floch, University of Brest, France
Infectious process and intraspecific diversity of *Colletotrichum lupini*, a fungal pathogen responsible for lupin anthracnose

14:50 Pedro Talhinhas, Universidade de Lisboa, Portugal
The olive anthracnose pathosystem as a case-study for fungal taxonomy, epidemiology and host-pathogen interactions towards sustainable disease resistance

15:15 Eduardo Goulin, Instituto Federal de Educação, Ciência e Tecnologia de Santa Catarina, Brazil
***Colletotrichum* and Citrus, the Postbloom fruit drop studies advances**

15:50 - 16:15 Coffee break

16:15 Peter Plaumann, Friedrich-Alexander-Universität Erlangen-Nürnberg, Germany
To have or not to have: A dispensable chromosome enables host colonization in the pathosystem *Colletotrichum higginsianum – Arabidopsis thaliana*

16:35 Thaís Regina Boufleur, University of São Paulo, Brazil
Regulation of Glycine max and *Colletotrichum truncatum* gene expression during colonization

17:55 Joris Alkemade, Research Institute of Organic Agriculture (FiBL), Switzerland
Genetic diversity within *Colletotrichum lupini*, the causal agent of lupin anthracnose, and its virulence on white lupin (*Lupinus albus*)

17:15 - 17:30 Closure

Horizontal gene transfer contributes to virulence in *Colletotrichum*

Michael R. Thon[1,*], José M. Sanz-Martín[1], Riccardo Baroncelli[1] and Serenella A. Sukno [1]

[1] Universidad de Salamanca, Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Villamayor, Salamanca, Spain

* presenting author: mthon@usal.es

Horizontal gene transfer (HGT) is the stable transmission of genetic material between organisms by means other than vertical inheritance. HGT has an important role in the evolution of prokaryotes but is relatively rare in eukaryotes. We studied the importance of HGT in plant pathogenic fungi by identifying horizontally transferred genes in the genomes of members of the genus *Colletotrichum*. We identified eleven HGT events from bacteria and one from plants to *Colletotrichum* spp. or their ancestors. The horizontally transferred genes from bacteria encode proteins involved in amino acid, lipid and sugar metabolism as well as lytic enzymes. Four of the eleven genes have homology to known virulence factors, suggesting that HGT may be important for niche adaptation and virulence. The putative minimal dates of the HGT events were calculated using a time calibrated phylogenetic tree, revealing a constant flux of genes from bacteria to fungi throughout the evolution of subphylum Pezizomycotina. HGT appears to be a constant, albeit rare phenomenon in the Pezizomycotina, occurring at a steady rate during their evolution. The gene acquired from plants encodes a protease which we call CPLS (*Colletotrichum* plant-like serine protease). Pathogenicity assays of CPLS null mutants show that CPLS is expressed at the early stages of pathogenesis and has a role in virulence. Transcriptional profiling shows that several independent defense mechanisms are suppressed in the presence of CPLS including NLR receptors, phenylpropanoid and flavonoid biosynthetic pathways. Thus, CPLS contributes to virulence by modulating host defense responses to promote plant susceptibility. Our results suggest that HGT is an important evolutionary process in fungi that contributes to the evolution of plant pathogens.

Genome rearrangements drive evolution of virulence-related genes in the genomes of *Colletotrichum gloeosporioides* species complex

Gan Pamela[1,*], Hiroyama Ryoko[1], Tsushima Ayako[1,2], Masuda Sachiko[1], Kumakura Naoyoshi[1], Hoat Trinh Xuan[3], Suzuki Takeshi[4], Narusaka Mari[5], Narusaka Yoshihiro[5], Takano Yoshitaka[6] and Shirasu Ken[1,2]

[1] RIKEN Center for Sustainable Resource Sciences, Yokohama, Kanagawa, Japan
[2] Graduate School of Science, The University of Tokyo, Bunkyo, Tokyo, Japan
[3] Plant Protection Research Institute, Ha Noi city, Vietnam
[4] Chiba Prefectural Agriculture and Forestry Research Center, Chiba, Japan
[5] Research Institute for Biological Sciences, Okayama Prefectural Technology Center for Agriculture, Forestry, and Fisheries, Okayama, Japan
[6] Graduate School of Agriculture, Kyoto University, Kyoto, Japan

* presenting author: pamela.gan@psc.riken.jp

Members of the *Colletotrichum gloeosporioides* species complex are causal agents of anthracnose in a wide range of commercially important plants. We sequenced the genomes of fungi from this species complex, *C. fructicola* and *C. siamense,* as well as representatives of three previously unsequenced species, *C. aenigma, C. tropicale* and *C. viniferum,* providing an in-depth overview of its diversity. Comparisons between multiple *C. fructicola* and *C. siamense* isolates led to the identification of large-scale, strain-specific genomic rearrangements and segmental duplications/loss in these genomes. Accessory regions present in *C. fructicola, C. siamense* and *C. aenigma* were found to be associated with secondary metabolite and effector candidate genes, which may contribute to host virulence. Analysis of near chromosomal-level assemblies of four isolates from these species reveal the presence of such accessory regions in sub-telomeric repeat-rich regions and in putative repeat-rich chromosomes, with exchange of genetic sequences occurring between such regions independently in different strains. Together, our results contribute to the understanding of genome evolution in the *Colletotrichum gloeosporioides* species complex.

Colletotrichum species diversity on aromatic and ornamental plant hosts in Italy.

Guarnaccia Vladimiro[1,2], Gilardi Giovanna[2], Martino Ilaria[2], Gullino Maria Lodovica[1,2] and Garibaldi Angelo[1,2]

[1] DiSAFA, University of Torino, Largo Paolo Braccini, 2, 10095 Grugliasco, TO, Italy
[2] Center of Competence Agroinnova, University of Torino, Largo Paolo Braccini, 2, 10095, Grugliasco, Italy

* presenting author: vladimiro.guarnaccia@unito.it

Species of *Colletotrichum* are considered important plant pathogens, saprobes, and endophytes on a wide range of plant hosts. In Italy, several *Colletotrichum* species have been reported in open field and greenhouse environment. In this study, the occurrence, diversity and pathogenicity of *Colletotrichum* spp. associated with aromatic belonging to the family Lamiaceae and several ornamental plants were explored. Surveys were carried out during 2011-2019 in Northern Italy. A total of 45 *Colletotrichum* strains were isolated from symptomatic leaves and twigs of 14 host species. Two multi-locus phylogenies were established based on four genomic loci (ITS, *GAPDH*, *ACT* and *TUB2*). Preliminary pathogenicity tests were performed with representative isolates. *Colletotrichum* strains were identified as members of four major species complexes: *C. acutatum*, *C. dematium*, *C. destructivum* and *C. gloeosporioides*. Totally, ten *Colletotrichum* species were found in association with leaf or twig lesions on the investigated hosts. The pathogenicity of one representative isolate for each combination *Colletotrichum* species/host was tested on seedlings or rooted cuttings kept in a growth chamber. All the tested strains were pathogenic and reproduced symptoms identical to those observed in natural conditions. The present study improves our understanding of *Colletotrichum* species associated with several hosts largely cultivated in Italy, and provides useful information for effective disease management.

Glycosylated flavonoids - fruit hidden arsenal against fungal pathogens

Kumar S Pradeep[1], Oleg Feygenberg[1], Dalia Maurer[1] and Noam Alkan[1,*].

[1] Department of Postharvest Science, Agricultural Research Organization, the Volcani Center, Israel

* presenting author: noamal@agri.gov.il

Fruit defense against pathogens relies on induced and preformed mechanisms. In this work, we evaluated performed resistance of red and green mango fruit against the fungal pathogen *Colletotrichum gloeosporioides* and identified the main active antifungal components. HPLC analysis of non-hydrolyzed mango peel extracts identified major glycosylated-anthocyanin and glycosylated-flavonol, which were more abundant on the 'red side' of red mango fruit. Organic extracts of red mango peel were more efficient in inhibiting *C. gloeosporioides* than green mango peel. Transcriptome analysis of the mango – *C. gloeosporioides* interaction showed increased expression of *glucosidase* genes related to both fungal pathogenicity and host defense. Glucosidase treatment on organic peel extract increased its antifungal activity. Additionally, quercetin and cyanidin had significantly higher antifungal activity than their glycosylated derivatives. The volatiles of peel extract treated with glucosidase had antifungal activity and included 15 volatiles, 7 of them present only in red fruit. These results suggest that the fruit obtains a concealed arsenal of glycosylated flavonoids in its peel when they are hydrolyzed by *β-glucosidase* that is induced by both fungus and host during infection process, they become more toxic to the fungal pathogen, inhibiting decay development.

Deficiencies in the mitochondrial electron transport chain affect redox poise and resistance towards *Colletotrichum higginsianum*

McCollum Christopher[1], Geißelsöder Sonja[1], Engelsdorf Timo[2], Voitsik Anna-Maria[1] and Voll Lars[2,*]

[1] Division of Biochemistry, Department Biology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany
[2] Molecular Plant Physiology, Department of Biology, Philipps-University Marburg, Marburg, Germany

* presenting author: lars.voll@biologie.uni-marburg.de

To investigate if and how the integrity of the mitochondrial electron transport chain (mETC) influences susceptibility of Arabidopsis towards *C. higginsianum*, we have selected previously characterized mutants with defects at different stages of the mETC, namely the complex I mutant ndufs4, the complex II mutant sdh2-1, the complex III mutant ucr8-1 and a mutant of the uncoupling protein, ucp1-2. Relative to wild type, the selected complex I, II and III mutants showed decreased total respiration, increased alternative respiration as well as increased redox charge of the NADP(H) pool and decreased redox charge of the NAD(H) pool in the dark. In the light, mETC mutants accumulated free amino acids, albeit to varying degrees. Glycine and serine, which are involved in carbon recycling from photorespiration, and N-rich amino acids were predominantly increased in mETC mutants compared to the wild type. Taking together the physiological phenotypes of all examined mutants, our results suggest a connection between the limitation in the re-oxidation of reducing equivalents in the mitochondrial matrix and the induction of nitrate assimilation into free amino acids in the cytosol, which seems to be engaged as an additional sink for reducing power. Taking metabolite profiling data from all investigated mETC mutants, a strong positive correlation of resistance towards *C. higginsianum* with NADPH pool size, pyruvate contents and other metabolites associated with redox poise and energy charge was evident, which fosters the hypothesis that limitations in the mETC can support resistance at post-penetration stages by improving the availability of metabolic power.

Investigating the role of a fungal oxidase-peroxidase tandem in plant pathogenicity

Bastien Bissaro[1,*], Sayo Kodama[2], Mireille Haon[1], David Ribeaucourt[1,3], Michael Lafond[3], Harry Brumer[4], Richard O'Connell[5], Yasuyuki Kubo[2] and Jean-Guy Berrin[1]

[1] INRA, Aix-Marseille Université, UMR Biodiversité et Biotechnologie Fongiques, Polytech'Marseille, Avenue de Luminy, Marseille, France
[2] Graduate School of Life and Environmental Sciences, Kyoto Prefectural University, Kyoto, Japan
[3] Aix Marseille Univ., CNRS, Centrale Marseille, iSm2, Marseille, France
[4] Michael Smith Laboratories, University of British Columbia, East Mall, Vancouver, Canada
[5]INRA, UMR BIOGER, AgroParisTech, Université Paris-Saclay, Thiverval-Grignon, France

* presenting author: bastien.bissaro@inra.fr

New members of the Copper-Radical Oxidases (CROs), from the Auxiliary Activity family 5_2 of the Carbohydrate-Active enZymes classification (CAZy – AA5_2, www.cazy.org), were recently found to catalyze the oxidation of aliphatic and aromatic alcohols to their corresponding aldehydes (Yin et al. 2015). Strikingly, these enzymes (named "AlcOx") are almost exclusively found in phytopathogenic ascomycete fungi and their biological function remains unknown. To tackle this question, using biochemical and biological approaches, we investigated the function of the AlcOx from different *Colletotrichum* species. At the transcriptomic level, analyses of *C. orbiculare, C. fructicola and C. graminicola* during infection revealed co-regulation of a pair of adjacent genes coding for an AlcOx and a putative peroxidase, only during the *appressorium* phase. At the enzyme level, work done on other CROs families point at an activating role of peroxidases on AlcOx, *via* a mechanism that remains to be elucidated. Recombinant production of AlcOx and peroxidases from *Colletotrichum* species is in progress to biochemically characterize their substrate preference and degree of synergism *in vitro*. At the biological level, the function of the AlcOx and peroxidase from the cucumber anthracnose fungus *C. orbiculare* is currently investigated *in vivo*. Preliminary results show that the pathogenicity of knock-out mutants decreased compared to the wild type strain, suggesting a role of the AlcOx and the peroxidase in the cucumber infection process by *C. orbiculare*.

Yin, D., Urresti, S., Lafond, M., Johnston, E.M., Derikvand, F., Ciano, L., Berrin, J.-G., Henrissat, B., Walton, P.H., Davies, G.J., Brumer, H., 2015. Structure–function characterization reveals new catalytic diversity in the galactose oxidase and glyoxal oxidase family. *Nature Communications* 6. https://doi.org/10.1038/ncomms10197

The effect of fruit sugar level on the pathogenicity mechanism and host response during *Colletotrichum* infection of red tomatoes

Carmit Ziv[1,*], Dilip Kumar[1], Arthur A. Schaffer[2] and Dov Prusky[1]

[1] Department of Postharvest Science, Agricultural Research Organization, the Volcani Center, Israel
[2] Plant Sciences, Vegetable and Field Crops, Agricultural Research Organization, the Volcani Center, Israel

* presenting author: carmit.ziv@volcani.agri.gov.il

The destructive phytopathogen *Colletotrichum* causes postharvest losses and secrete ammonia, as a mechanism to modulate environmental pH and regulate gene expression that contribute to differential pathogenicity. Previous observations indicated that the differential pH modulation is strongly dependent on carbon availability causing acidification or alkalization of the host tissue in the presence of carbon excess or its limitation, respectively. The natural increase in total soluble solids (TSS) in ripening fruits suggested a dynamic process of pH modulation occurring in harvested fruit. In the present work the response of two near isogenic tomato lines, contributing to the differential level of TSS content, were compared. *C. gloeosporioides* showed enhanced colonization of the LowSC (Sugar Content) line compared to the colonization of the HighSC. Fungal genes showed enhanced frequency of macromolecules metabolic processes and decrease in the frequency of carbohydrate metabolic process. Under conditions of enhanced colonization in the LowSC, the expression of glycosyl hydrolases, glucanases and the activation of several MSF transporters by *C. gloeosporioides* were observed. However, the host response of reduced colonized HighSC line by *C. gloeosporioides* was accompanied by an increase expression of glucosyltransferases with UDP-glucosyltransferase activity, and transferases transferring glycosyl groups genes of importance in the induction of resistance by regulation of plant defense against pathogens. While the increased colonization includes the activation of specific pathogenicity factors by the pathogen, the level of sugar present in the host may as well modulate fungal colonization that differentially activate the fungitoxic activity of phenylpropanoid metabolism resulting in compounds affecting defense against pathogens.

Infectious process and intraspecific diversity of *Colletotrichum lupini*, a fungal pathogen responsible for lupin anthracnose

Le Floch Gaetan[1,*], Dubrulle Guillaume[1], Pensec Flora[1], Picot Adeline[1], Nodet Patrice[1], Madec Stephanie[1] and Baroncelli Riccardo[2]

[1] University of Brest, Laboratoire Universitaire de Biodiversité et Ecologie Microbienne, ESIAB, F-29280 Plouzané, France
[2] Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), University of Salamanca, Calle 10 del Duero, 12; 37185 Villamayor (Salamanca), Spain

* presenting author: gaetan.lefloch@univ-brest.fr

Lupin anthracnose is a major threat caused by the filamentous fungus *Colletotrichum lupini*. The genus *Colletotrichum* includes a wide number of phytopathogen species distributed worldwide. They were classified into 14 species complexes, with *C. lupini* belonging to the *C. acutatum* species complex. To date, studies performed on *C. lupini*, aimed at better understanding its phylogenetic classification and infectious lifecycle. But additional studies seemed necessary to increase our knowledge on (i) intraspecific diversity within the *C. lupini* species and (ii) the molecular determinants of pathogenicity. To address the first objective, a study was performed on strains from official collections and from isolates recently collected in the west of France. A low intra-specific phylogenetic diversity was found compared to the phenotypic diversity, notably characterized by contrasted aggressiveness between strains. The second objective was evaluated by determining fungal gene expression and protein synthesis during lupin infection by *C. lupini* using, respectively, a RNAseq-based transcriptomic approach and a mass spectrometry-based proteomic approach with a nLC Q-exactive Orbitrap. Taken together, our results highlighted that the dynamics of symptoms, gene expression and protein synthesis shared similarities to those of hemibiotrophic pathogens. In addition, few genes of unknown or poorly-described functions were found to be specifically associated to the early or late stages of infection, suggesting that they may be of importance for pathogenicity. Functional validation will be discussed to confirm their role in promoting *C. lupini* pathogenicity.

The olive anthracnose pathosystem as a case-study for fungal taxonomy, epidemiology and host-pathogen interactions towards sustainable disease resistance

Talhinhas Pedro[1,*], Loureiro Andreia[1] and Oliveira Helena[1].

[1] LEAF, Instituto Superior de Agronomia, Universidade de Lisboa. Tapada da Ajuda, 1349-017 Lisboa, Portugal

* presenting author: ptalhinhas@isa.ulisboa.pt

Colletotrichum fungi are interesting models to study fungal biology. However, many of the economically-relevant diseases caused by *Colletotrichum* occur on perennial hosts, often with limited availability of target organs due to specificities of the host life cycle. Far from easy or fast, nevertheless such pathosystems are real-life cases that require attention and may provide important information. The olive anthracnose pathosystem is such an example. Olive anthracnose shows up on olive fruits as they mature, causing fruit drop (yield loss) or deteriorated oil, with the pathogen persisting mostly asymptomatically on leaves and branches until fruits mature in the next autumn. Dissecting the olive-*Colletotrichum* interaction using histopathology and epidemiology tools has enabled a more detailed characterization of this pathosystem, prompting crop protection strategies. Additionally, olive anthracnose pathogens are diverse, with least 12 genetically distinct fungal populations causing olive anthracnose, and have in part contributed to the current taxonomic framework of the acutatum species complex, where most of them cluster. While *Colletotrichum nymphaeae* and *C. acutatum* s.s. are the most virulent, the occurrence of interaction in virulence levels between *Colletotrichum* spp. and olive cultivars have been identified, prompting the need for further selection studies. Species of *Colletotrichum* causing olive anthracnose vary according to geography. Recent data suggests that *C. acutatum* s.s. may be replacing the less virulent *C. godetiae* in the central Mediterranean area, thus representing a regional scale epidemiology case study. Olive production, harvest and processing systems are experiencing profound changes and stricter rules concerning pesticide use are likely to have a strong impact on control strategies. A detailed knowledge of pathogen diversity, population dynamics and host-pathogen interactions is thus basal for the deployment of durable and effective disease control strategies.

Colletotrichum and Citrus, the Postbloom fruit drop studies advances.

Goulin Eduardo H.[1,2,*] and Machado Marcos A.[2]

1 Instituto Federal de Educação, Ciência e Tecnologia de Santa Catarina, Canoinhas, Santa Catarina, Brazil
2 Centro de Citricultura "Sylvio Moreira" (CCSM), Instituto Agronômico (IAC), Cordeirópolis, São Paulo, Brazil.

* presenting author: eduardo.goulin@gmail.com

Since ancient times citrus is an economically important culture for countries worldwide. Phytopathogens are responsible for significant losses in citrus production and fruit quality. Postbloom fruit drop (PFD) is one of the fungal diseases that can attack the citrus crop. The disease is characterized by lesions on the petals that can extend to young fruits, inducing their early drop. One of the causal agents of this disease is *Colletotrichum abscissum*, which belongs to the *Colletotrichum acutatum* species complex. Over the past years, there were several changes in the taxonomy of *Colletotrichum* species. To illustrate that, one species that cause disease in citrus named *C. acutatum* was divided in 32 different species, many of them have their genomes and transcriptome sequenced, which is an advantage for its studies. The control of PFD is based on chemical applications that can induce the resistance development over the time, besides other parallel damages caused by fungicides. New technologies are being applied every year to better understand the pathogens biology, moreover they contribute to plant diseases control as an alternative to chemical one, such as the RNA interference technology. Furthermore, the mechanisms to test the new control strategies became necessary. In this scenario, many advances were performed to address answers to those questions. We compared our isolates from PFD lesion in citrus with all type strains from *C. acutatum* complex (described by Damm), and all isolates from PFD that affect flowers of sweet orange, as well as our isolates in Brazil, that belongs to the species of *C. acutatum* complex named *C. abscissum*. Irrespective of crops and fungicides used, development of fungicide- resistant pathogen populations is among the most severe problems in agriculture. Therefore, we investigate the functionality of the RNAi machinery in *C. abscissum* and test genetically whether the chemically pre-defined fungal SDHi target may represent a promising target gene in host-induced gene silencing (HIGS) plants. The *C. abscissum* RNAi machinery was functionally proven by silencing of gene report. Then, the silencing of SDH subunits were induced and verified. the RNA interference is an important tool that can be exploited to post bloom fruit drop disease control and also the chemical fungicide target are still useful in the new technologies control strategies. We developed a reliable alternative *in vitro* system for symptom induction of *C. abscissum* infection in detached citrus flowers. Inoculated citrus flowers were placed on Petri dishes with a water-agar substrate and typical symptoms of PFD after 72 hours in BOD chambers. This is an undoubtedly effective large-scale screening system, especially because it is fast and reliable and requires less space than do greenhouses or open field experiments. These previous results led us to test and successfully silence the fungal genes by double-strand RNAi. And, more recently, HIGS plants were generated and will be tested for gene silencing efficiency against *C. abscissum*. In conclusion, it is notable that advances with the PFD pathogen and promising control strategies will be available during the next feel years.

To have or not to have: A dispensable chromosome enables host colonization in the pathosystem *Colletotrichum higginsianum* – *Arabidopsis thaliana*.

Plaumann Peter-Louis[1], Satzinger Evelina[1], Dahl Marlis[1] and Koch Christian[1].

[1] Division of Biochemistry, Department of Biology, Friedrich-Alexander-Universität Erlangen-Nürnberg, Erlangen, Germany

* presenting author: peter-louis.plaumann@fau.de

Colletotrichum higginsianum is a hemibiotrophic plant pathogen whose hosts are different members of the Brassicaceae family. Together with Arabidopsis thaliana, it represents a prominent model system to investigate various ecologically important fungal pathogens and their infection strategies. The infection cycle starts with the mechanical penetration of the plant cell enabled by specialized cells called appressoria. Subsequently, *C. higginsianum* establishes large biotrophic primary hyphae in the first infected cell. Shortly thereafter a switch to necrotrophic growth occurs, leading to the invasion of neighboring cells by secondary hyphae.
We characterized a dispensable mini chromosome (chr11) enriched with effector genes that is essential for virulence on A. thaliana. *C. higginsianum* strains lacking chromosome 11 (chr11Δ) do not show any obvious vegetative defects but are not able to switch from biotrophy to necrotrophy during infection. Analysis of plant defense mutants showed that genes encoded on chromosome 11 are required to suppress PAMP triggered immunity especially the production of tryptophan derived secondary metabolites. By comparative genomics and karyotype analysis of different fungal isolates, we identified genetic variations between mini chromosomes. This enabled us to identify the region on chromosome 11 whose presence correlates with successful *A. thaliana* infection. We further present genetic analysis of this region which allowed us to identify important virulence factors necessary for necrotrophic colonization of the host plant.

Colletotrichum truncatum effector repertoire revealed by comparative genomics and transcriptomics analyses

Thaís Regina Boufleur[1], Nelson Sidnei Massola Júnior[1], Flávia Rogério[1], Maísa Ciampi-Guillardi[1], Serenela Ana Sukno[2], Michael Ronald Thon[2], Riccardo Baroncelli[2].

[1] Department of Plant Pathology and Nematology, University of São Paulo, Piracicaba, SP, Brazil; and
[2] Universidad de Salamanca, Instituto Hispano-Luso de Investigaciones Agrarias (CIALE), Villamayor, Salamanca, Spain.

* presenting author: thaisboufleur@usp.br

Soybean anthracnose is mainly associated with the hemibiotrophic fungus Colletotrichum truncatum, but recently several new species of Colletotrichum have been reported in commercial soybean fields. In order to better understand the adaptation of anthracnose causing Colletotrichum spp to soybean we sequenced and analyzed the genome of 4 representative isolates belonging to the pathogenic species C. truncatum, C. plurivorum, C. sojae and C. musicola. The genomes were assembled, and gene prediction and annotation were performed to identify and characterize protein encoding genes. The proteomes and secretomes of the newly sequenced genomes along with those of an additional 8 species representing the diversity of the genus and not pathogenic to soybean were classified into protein families using a variety of bioinformatic approaches. Comparative genome analyses of small secreted proteins identified complex-specific and species-specific proteins but did not identify proteins that are shared only between the 4 soybean-pathogenic species. Identified lineage specific effector protein candidates (LSECs) where further characterized by the predicted localization of the mature peptides. A transcriptomic analysis was carried out at different timepoints of infection to investigate the expression profiles of C. truncatum genes in planta. Based on these analyses, four species specific effector protein candidates (SSECs) were identified that are predicted to be secreted, targeting the nucleus overexpressed in planta at 12 hours post inoculation (early-penetration stage). These specific extracellular proteins may be effectors, proteins that have important roles in modulating the plant's immune system and in host specificity. These results represent a new resource that will be useful for further research into the biology and evolution of these key pathogens and in the management of soybean anthracnose.

Genetic diversity within *Colletotrichum lupini,* the causal agent of lupin anthracnose, and its virulence on white lupin (*Lupinus albus)*

J. Alkemade[1,2,*], M. M. Messmer[1], R. T. Voegele[2], M. R. Finckh[3], P. Hohmann[1]

1 Department of Crop Sciences, Research Institute of Organic Agriculture (FiBL), Switzerland
2 Institute of Phytomedicine, University of Hohenheim, Germany
3 Department of Ecological Plant Protection, Kassel University, Germany

* presenting author: joris.alkemade@fibl.org

White lupin (*Lupinus albus*) is a grain legume known for its high protein content and quality, efficient nutrient acquisition and health benefits (Lambers et al. 2013, Arnoldi et al. 2015). Its high yield potential could make it a sustainable alternative to soybean in cooler regions (Lucas et al. 2015). However, since the 1980s anthracnose disease, caused by the air- and soil-borne fungal disease *Colletotrichum lupini*, threatens lupin cultivations worldwide (Nirenberg et al. 2002, Damm et al. 2012, Talhinhas et al. 2016). Even low levels of seed infestation can lead up to total yield loss (Thomas 2004, Diggle 2002). To assist white lupin breeding programs, we analyzed the genetic diversity of globally collected lupin-infecting *Colletotrichum* isolates by multi-locus sequencing (Pecchia et al. 2019, Dubrulle et al. 2019). First analyses indicate that all isolates belong to the species *C. lupini* and that the genetic diversity of isolates collected from Europe and Australia is lower compared with isolates collected from the South American Andes, showing different genetic groups. An indoor screening assay was developed and validated by field performance, allowing to determine differences in virulence between *C. lupini* strains under controlled conditions. Currently, virulence tests of selected *C. lupini* isolates are being performed on two white lupin cultivars, the susceptible variety Feodora and the tolerant breeding line Blu-25 from Erik von Baer. Our study will shed light on the genetic makeup of the species *C. lupini* and its relation to virulence on white lupin, thereby providing valuable information to improve white lupin breeding programs.

Arnoldi, A., Boschin, G., Zanoni, C. & Lammi, C. 2015. The health benefits of sweet lupin seed flours and isolated proteins. *Journal of Functional Foods,* 18**,** 550-563.

Damm, U., Cannon, P. F., Woudenberg, J. H. C. & Crous, P. W. 2012. The Colletotrichum acutatum species complex. *Studies in Mycology,* 73**,** 37 - 113.

Dubrulle, G., Pensec, F., Picot, A., Rigalma, K., Pawtowski, A., Nicolleau, S., Harzic, N., Nodet, P., Baroncelli, R. & Le Floch, G. 2019. Phylogenetic Diversity and Effect of Temperature on Pathogenicity of Colletotrichum lupini. *Plant Disease***,** 1-24.

Lambers, H., Clements, J. C. & Nelson, M. N. 2013. How a phosphorus-acquisition strategy based on carboxylate exudation powers the success and agronomic potential of lupines (Lupinus, Fabaceae). *American Journal of Botany,* 100**,** 263-288.

Lucas, M. M., Stoddard, F. L., Annicchiarico, P., Frias, J., Martinez-Villaluenga, C., Sussmann, D., Duranti, M., Seger, A., Zander, P. M. & Pueyo, J. J. 2015. The future of lupin as a protein crop in Europe. *Frontiers in Plant Science,* 6**,** 705-711.

Nirenberg, H. I., Feiler, U. & Hagedorn, G. 2002. Description of Colletotrichum lupini comb. nov. in modern terms. *Mycologia,* 94**,** 307-320.

Pecchia, S., Caggiano, B., Da Lio, D., Cafà, G., Le Floch, G. & Baroncelli, R. 2019. Molecular Detection of the Seed-Borne Pathogen Colletotrichum lupini Targeting the Hyper-Variable IGS Region of the Ribosomal Cluster. *Plants,* 8**,** 222-238.

Talhinhas, P., Baroncelli, R. & Le Floch, G. 2016. Anthracnose of lupins caused by Colletotrichum lupini: a recent disease and a successful worldwide pathogen. *Journal of Plant Pathology,* 98**,** 5-14.

Oral presentation @ECFG15

Tuesday the 18th of February 2020
Molecular taxonomy & phylogenomics (CS 3.2)

16:30-16:45 Michael Thon, University of Salamanca, Spain
Horizontal gene transfer contributes to virulence in *Colletotrichum*

Wednesday the 19th of February 2020
Genome, chromatin and epigenetics (CS 1.4)

17:30-17:45 Riccardo Baroncelli, University of Salamanca, Spain
Genome analyses reveal evolution and adaptation of carbohydrate utilization in the genus *Colletotrichum*