

MCFARLANE Dylan. (14,15), MATTNER Scott. (14,15), GAMBARDELLA Marina. (16), HENRY Peter M. (1)

(1) United States Department of Agriculture, Agricultural Research Service, Salinas, UNITED STATES; (2) University of California, Davis, Davis, UNITED STATES; (3) Universidad de Sevilla, Sevilla, SPAIN; (4) Queensland Department of Agriculture and Fisheries, Brisbane, AUSTRALIA; (5) Michigan State University, East Lansing, UNITED STATES; (6) Christian-Albrechts University of Kiel, Kiel, GERMANY; (7) Max Planck Institute for Evolutionary Biology, Plön, GERMANY; (8) University of São Paulo, Ribeirão Preto, UNITED STATES; (9) Technical University of Munich, Munich, GERMANY; (10) Universidade Federal de Pernambuco, Recife, BRAZIL; (11) United States Department of Agriculture, Agricultural Research Service, Jackson, UNITED STATES; (12) University of Florida - Gulf Coast Research and Extension Center, Wimauma, UNITED STATES; (13) Driscoll's, Inc., Watsonville, UNITED STATES; (14) VSICA Research, Croydon, AUSTRALIA; (15) La Trope University, Melbourne, AUSTRALIA; (16) Pontificia Universidad Católica de Chile, Santiago, CHILE

### Text

*Macrophomina phaseolina* has a broad host range, but individual isolates may be pathogenic to a limited number of hosts. Previous work demonstrated: 1) strawberry is not susceptible to all isolates of the fungus, and 2) host specialization may have occurred among highly aggressive, strawberry-pathogenic isolates. A survey of the genus was conducted to identify host-genotype associations, pangenomic structure and mechanisms of genetic exchange. Short-read sequence data were obtained for 422 *Macrophomina* spp. isolates collected from 94 host plant species in 27 countries (113 from strawberry, 54 from soybean, 255 from other hosts). High-quality short-reads were assembled and mapped to reference genomes. *M. phaseolina* was grouped into ten partially recombinant clades, with high admixture in some isolates suggesting on-going recombination. Three of the identified clades clustered with respect to isolate's host of origin (78% of strawberry-derived isolates were in a single clade, whereas 74% of soybean isolates resolved into two clades). This pattern suggests that host specialization may be occurring among isolates in specific clades. Furthermore, pathogenicity tests of select isolates representing each clade suggest strawberry is only highly susceptible to isolates from the "strawberry clade". *Macrophomina* appears to have a one-speed genome. This work provides insight into host specialization and evolutionary mechanisms within this economically important pathogen genus.

### C4.3-3

## ADAPTIVE EVOLUTION IN VIRULENCE EFFECTORS OF THE RICE BLAST FUNGUS *PYRICULARIA ORYZAE*

LE NAOUR VERNET Marie. (1), CHARRIAT Florian. (2), GRACY Jerome. (3), CROS-ARTEIL Sandrine. (1), RAVEL Sebastien. (2), MEUSNIER Isabelle. (1), PADILLA André. (3), KROJ Thomas. (1), CESARI Stella. (1), **GLADIEUX Pierre. (1)**

(1) INRAE, Montpellier, FRANCE; (2) CIRAD, Montpellier, FRANCE; (3) CNRS, Montpellier, FRANCE

### Text

Plant pathogens secrete proteins called effectors that target host cellular processes to promote disease. Recently, structure-based clustering has identified several families of fungal effectors that share a conserved three-dimensional structure despite remarkably

variable amino-acid sequences and surface properties.

To explore the selective forces that underlie the sequence variability of structurally-analogous effectors, we focused on MAX effectors, a structural family of effectors that are major determinants of virulence in the rice blast fungus *Pyricularia oryzae*. Using structure-informed gene annotation, we identified 58 to 78 MAX effector genes per genome in a set of 120 isolates representing seven host-associated lineages. The expression of MAX effector genes was primarily restricted to the early biotrophic phase of infection and strongly influenced by the host plant. Pangenome analyses of MAX effectors demonstrated extensive presence/absence polymorphism and identified several candidate gene loss events possibly involved in host range adaptation. MAX effectors displayed high levels of standing variation and high rates of non-synonymous substitutions, pointing to widespread positive selection shaping their molecular diversity.

Our work demonstrates that MAX effectors represent a highly dynamic compartment of the genome of *P. oryzae*, and suggests that MAX effectors are key players in molecular coevolutionary interactions with plant hosts.

#### C4.3-4

### **PATHOGENICITY OF THE CONIFER WILT PATHOGEN, LEPTOGRAPHIUM WAGENERI: GENOMIC INSIGHTS**

DU PLESSIS Deanne. (1), WINGFIELD Brenda. (1), ENGELBRECHT Juanita . (1), VANG QUY Le. (2), WINGFIELD Mike. (1), **DUONG Tuan. (1)**

(1) University of Pretoria, Pretoria, SOUTH AFRICA; (2) Aalborg University Hospital, Aalborg, DENMARK

#### **Text**

*Leptographium wagneri* is an ascomycete fungal pathogen that causes black stain root disease (BSRD) of conifers. As a primary pathogen that has evolved from closely related saprotrophic species, *L. wagneri* offers an opportunity to identify the possible determinants of pathogenicity. We sequenced the genomes of the three varieties of *L. wagneri* and the closely related non-pathogenic *L. douglasii* and performed comparative genomics between the pathogens and closely related non-pathogenic species. The three varieties of *L. wagneri* were found to have larger genomes, higher gene numbers and a higher content of transposable elements. A putative laccase gene was present only in the three varieties of *L. wagneri* and *L. douglasii*. This laccase gene was horizontally acquired by the common ancestor of *L. wagneri* and *L. douglasii* and encodes for a secreted laccase. Infection of *P. patula* seedlings followed by qRT-PCR analysis indicated that this laccase gene was upregulated in-plant, suggesting its role in pathogenicity. Subsequently, laccase knockout mutants were generated using CRISPR-Cas9 and used in a pathogenicity test. The results showed that the laccase-deleted mutants failed to cause typical symptoms of infection by *L. wagneri*. Collectively, this study illustrates patterns of genome evolution in *L. wagneri* from a non-pathogenic relative. The results also provide some evidence that a horizontally acquired laccase is a key virulence factor in this tree pathogen.