Oral Technical Session: Pathogen Resistance

162-O

Monitoring for resistance in *Botrytis cinerea* from strawberry to seven chemical classes of fungicides in the eastern United States.

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Chemical control of gray mold of strawberry caused by *Botrytis cinerea* Pers. is essential to prevent pre- and postharvest fruit decay; however, resistance to multiple chemical classes of fungicides including anilinopyrimidines (APs), dicarboxamides (DCs), hydroxyanilides (HAs), methyl benzimidazole carbamates (MBCs), phenylpyrroles (PPs), quinone outside inhibitors (QoIs), or succinate dehydrogenase inhibitors (SDHIs) was found recently in *B. cinerea* from strawberry fields in the Carolinas. Resistance to DCs, HAs, MBCs, QoIs, and SDHIs, was caused by point mutations in the corresponding target genes (*Daf1*, *Erg27*, *b-tubulin, Cytb*, and *SdhB*); however, resistance mechanisms for APs and PPs are still unknown. A resistance-monitoring program was implemented to help growers determine location-specific resistance profiles. The analysis of samples from Arkansas, Florida, Georgia, Kansas, Maryland, North Carolina, South Carolina, and Virginia strawberry fields revealed *B. cinerea* isolates resistant to multiple chemical classes, including the PP fungicide fludioxonil, and to several SDHI fungicides not yet available for gray mold control. Our results indicate that resistance to some chemical classes including APs, HAs, MBCs, QoIs, and SDHIs is widespread and resistance DCs and PPs is emerging.

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tests. To map the resistance gene(s) in PI 182103, 186 F_6 RILs were developed from a cross with Avocet S. The seedlings of the parents and F_1 and RIL progeny tested with PST-100 and PST-114 indicated that PI 182103 had three recessive genes for resistance. In 2011 and 2012, the parents and RILs were evaluated in randomized field experiments at Pullman and Mt. Vernon, Washington with three replications at each location. Infection type and disease severity (DS) data were recorded three times for each line. Both IT and DS-based area under the disease progress curve (AUDPC) data indicated that the PI 182103 resistance to stripe rust is controlled by quantitative trait loci (QTL). About 860 pairs of simple sequence repeat (SSR) primers were used to map the resistance QTL. Three QTL that were detected in the seedling stage were mapped on chromosome 2AS, 3AL and 4DL and two major QTL only detected in the field experiments were mapped on chromosomes 5BS and 7BL, which explained 23.2-30.1 and 23.1-58.2 of the phenotypic variation, respectively. Our results suggested that these genes in combination provide high and potentially durable resistance to stripe rust.

The coat protein of *Tobacco necrosis virus* acts elicits HR in *Nicotiana* species belonging to section *Alatae*
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We tested the capacity of *Tobacco necrosis virus* (TNV) to infect 21 *Nicotiana* species and found that virion inoculations triggered a hypersensitive response (HR) in all species except *N. benthamiana*. In the case of *N. benthamiana*, TNV moved systemically. To identify putative viral avirulence proteins responsible for eliciting HR, we agroinfiltrated TNV genes individually into *Nicotiana* species. To date, we have found that the TNV coat protein triggers HR in six members of section *Alatae*. These species include *N. langsdorffii*, *N. longiflora*, *N. bonariensis*, *N. alata*, *N. forgetiana* and *N. mutabilis*. The only member of section *Alatae* that failed to respond with HR to agroinfiltration of the TNV CP was *N. plumbaginifolia*. Mutational analysis of the TNV CP confirmed that the protein rather than the RNA sequence was responsible for HR elicitation. A previous study had shown that the TBSV CP is also an avr determinant for the same *Nicotiana* species in section *Alatae*. TNV and TBSV are related, as both are type species of their respective genera in the family Tombusviridae. An alignment of the CPs of TNV and TBSV reveals some similarities at the primary sequence level. Further research will be directed towards investigating whether sequences shared by the CPs of TNV and TBSV are responsible for recognition by a single host resistance protein or if host resistance proteins are able to distinguish between the CPs of TNV and TBSV.

Mechanisms of adaptation to host rice cells by the blast fungus
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To infect rice, the devastating blast fungus *Magnaporthe oryzae* has distinct morphogenetic stages that allow it to breach the surface of the host leaf and invade the plant tissue. How the fungus monitors the transition from the nutrient-free surface to the nutrient-rich interior of the leaf, what controls the genetic reprogramming necessary to produce infectious hyphae, and how it acquires nutrient during biotrophic in planta growth is poorly understood. *M. oryzae*'s trehalose-6-phosphate synthase 1 (Tps1) enzyme integrates carbon and nitrogen metabolism in the fungal cell to regulate virulence via a novel NADPH-dependent genetic switch. Loss of Tps1 function results in Δ*tps1* strains that can form functional appressoria and penetrate the rice surface but fail to grow beyond the first infected cell. Impaired invasive growth of Δ*tps1* strains is due to loss of glucose sensing, inactivation of the NADPH-dependent genetic switch, and altered carbon assimilation. Moreover, NADPH-requiring antioxidation systems are shut down in Δ*tps1* strains, rendering them hypersensitive to oxidative stress. Taken together, we are exploring the dynamics of this critical NADPH-dependent genetic switch to understand how *M. oryzae* controls infectious hyphal development during biotrophy.

Monitoring for resistance in *Botrytis cinerea* from strawberry to seven chemical classes of fungicides in the eastern United States
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Genetic variability suggests that *Ceratocystis fimbriata* is native to Rio Grande do Sul, Brazil, where it is causing a new wilt disease on kiwifruit
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Ceratocystis fimbriata is a native, soilborne pathogen in South America with a broad host range. Ceratocystis wilt on kiwifruit (*Actinidia deliciosa*) was first recognized in 2010 in the state of Rio Grande do Sul, Brazil. Affected plants show wilting and death of leaves, which hang on the branches, and reddish-brown staining in a radial pattern in the xylem. The disease reduces the number of harvestable fruit, and most affected plants die. We examined genetic variation in the pathogen population to see if there was only a single genotype, suggesting that the fungus had been introduced from elsewhere in infected nursery stock, or if there was substantial genetic variation, suggesting that the fungus was soilborne and indigenous to the region. We used 14 microsatellite markers to identify 16 genotypes of *C. fimbriata* among 76 isolates from individual trees in eight kiwi plantations. There was also significant genetic variation in ITS-rDNA, *MAT1-1-2*, and *MAT1-2-1* DNA sequences, which placed the fungus among other South American populations of *C. fimbriata*. One of the genotypes was found in seven plantations, including an area where grafted plants had been produced and sold to other growers. However, other isolates showed substantial genetic variability, indicating that the pathogen is native to Rio Grande do Sul. This is the southern-most report of *C. fimbriata* in Brazil, where the disease may prove to be a major limiting factor in kiwi production.

Identification and detection of wheat pathogens through volatile organic compound analysis
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Research shows that fungi produce different VOCs in different concentrations depending on their species, developmental stage, and abiotic and biotic factors. The objective of this work was to detect and distinguish fungal wheat pathogens by VOC profile analysis. Single spore isolates from *Fusarium graminearum*, *F. culmorum*, *F. poa*, *F. avenaceum*, *Phaeosphaeria nodorum*, *Mycosphaerella graminicola*, and *Pyrenophora tritici-repentis* were grown 7 days in 5cm-PDA petri dishes. In parallel experiments, wheat plants were inoculated with *B. graminis*, *F. graminearum*, and *P. nodorum* and sampled 7, 14 and 21 days post inoculation dpi. We collected VOCs using a Super Q filter fitted on airtight glass containers, eluted with hexane, and injected the extract into the GC/MS. The GC data sets were analyzed, aligned and compared using MetAlignTM. VOC collection of each isolate and each wheat-pathogen combination was repeated 3-5 times. In contrast to the controls, *P. nodorum* isolates produced Mellein, *M. graminicola* produced 6-methyl-5-hepten-2-one, Isosativene, 2-one, and Mellein, while *F. poa* produced 1,2,3-trimethylbennen and Sesquiterpen, possibly Germacrende D. Powdery mildew, leaf blotch and Fusarium head blight produced clearly distinguishable VOC profiles. We will discuss the application of these findings in early detection of plant diseases and in site specific disease control.

Stability of azoxystrobin resistance and fitness of fungicide-sensitive and -resistant field isolates of *Didymella bryoniae*
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Stability of azoxystrobin (AZO) resistance and fitness of fungicide-sensitive (S) and -resistant (R) isolates of *Didymella bryoniae* were investigated using

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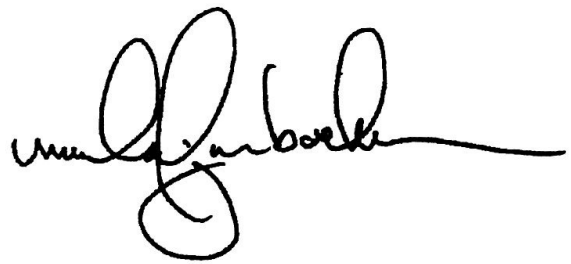

Michael Boehm, President