
# 2016 APS Annual Meeting
# Abstracts of Presentations

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**Factors that influence the structure of *Citrus tristeza virus* populations**
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A virus infection of a host is not a discrete, static entity, but a dynamic population whose members interact with one another and with their host to persist and spread. We have been using populations of *Citrus tristeza virus* (CTV) strains to examine this phenomenon, and have found that under constant conditions, the population structure will stabilize to an equilibrium defined by the host. This led us to ask what determinants, other than the host, can define a population, and what factors can cause a shift in population structure. Here we examined the effect of (a) inducing stress on the host through drought, high temperature, and by inducing systemic acquired resistance through the application of salicylic acid (SA), and (b) the addition of *Citrus tatter leaf virus* (CTLV) and *Citrus leaf blotch virus* (CLBV). We found that the structure of a CTV population comprised of three strains showed no significant change between treated and non-treated plants when exposed to drought or treated with SA, nor did the addition of CTLV or CLBV. Only exposure to approximate Florida summer temperatures (36°C day/25°C night) caused a change in structure, decreasing virus titer in a strain-specific and host-specific manner. Knowledge of the factors that determine population structure will aid in developing methods to manipulate populations and prevent disease.

**Virus populations associated with Maize lethal necrosis (MLN) in East Africa**
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Maize lethal necrosis (MLN) has emerged as a devastating disease in East Africa in recent years. MLN is caused by co-infection of a maize-infecting potyvirus and maize chlorotic mottle virus (MCMV). Deep sequencing of MLN-associated samples collected in Kenya and Uganda indicate high conservation of MCMV sequences, whereas the associated potyvirus population is diverse and complex. Sugarcane mosaic virus (SCMV)-like sequences are abundant and variable across samples, and clustered into four groups with over 10% nucleotide sequence divergence. Sequence analyses indicate presence of other putative viruses in MLN-diseased tissue that require further characterization. These data are valuable to inform diagnostics, containment, and management of destructive maize viruses in East Africa.

**Investigating the spread of Grapevine red blotch-associated virus**
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Red blotch is a recently recognized viral disease of *Vitis* spp. The causal agent, Grapevine red blotch-associated virus (GRBaV) has a single-stranded, circular DNA genome (3,206 nt), and is a proposed member of the family *Geminiviridae*. GRBaV has been detected in all major grape-growing regions of the United States and Canada, likely as a result of the dissemination of infected propagation and planting material. Of major concern to the grape industry is whether GRBaV is transmitted in vineyards. While there is no indication of spread in most areas where GRBaV has been detected, within-vineyard transmission is documented in California, likely due to insect vectors. To identify insect vectors of GRBaV, a detailed census of hemipteran insects in a selected California vineyard was performed. Of more than 50 species screened in 2015, only four species of the families Cicadellidae, Membracidae, and Cixiidae consistently carried genetic elements of GRBaV, as shown by multiplex PCR. The ability of vector candidate species to

Isolates of the *Colletotrichum acutatum* species complex were collected from South Carolina and Georgia peach orchards. Phylogenetic analysis of the combined ITS, glyceraldehyde-3-phosphate dehydrogenase, and beta-tubulin gene sequences separated the isolates into two species, *Colletotrichum nymphaeae* and *Colletotrichum fioriniae* subgroups 1 and 2. The sensitivity of these and three other *Colletotrichum* species from peach (*Colletotrichum fructicola, Colletotrichum siamense, and Colletotrichum truncatum*) to demethylation inhibitor (DMI) fungicides difenconazole, propiconazole, tebuconazole, metconazole, flutriafol, and fenbuconazole were determined. *C. truncatum* was resistant to tebuconazole, metconazole, flutriafol, and fenbuconazole. *C. nymphaeae* was resistant to flutriafol and fenbuconazole. *C. fructicola* and *C. siamense* were sensitive to all DMI fungicides, and *C. fioriniae* subgroup 2 was reduced-sensitive to DMI fungicides compared with *C. fioriniae* subgroup1. Difenconazole and propiconazole provided the best control efficacy in vitro to all five species. Tebuconazole and metconazole were effective against all *Colletotrichum* species, except for *C. truncatum*. The 14-α sterol demethylase genes *CYP51A* and *CYP51B* from resistant isolates revealed point mutations associated with resistance to DMI fungicides. The strong in vitro activity of some DMI fungicides against *Colletotrichum* species may be exploited for improved anthracnose disease management of peach.

**In-vitro azoxystrobin sensitivity of *Colletotrichum gloeosporioides* isolates from blueberry in north and central Florida**

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Anthracnose pathogens cause destructive diseases of blueberry fruits, leaves, and stems in Florida. Growers in central Florida have reported a lack of efficacy from QoI fungicides, specifically for anthracnose stem cankers on the southern highbush blueberry variety Flicker. Twenty-two single-spore isolates collected from symptomatic stems, leaves, and berries were identified and tested for sensitivity to QoI fungicides. Percent inhibition (PI) of conidial germination on PDA plates amended with 10µg $mL^{-1}$ of azoxystrobin demonstrated all isolates from central Florida farms were insensitive to azoxystrobin. Isolates from north Florida farms were sensitive. Internal transcribed spacer region (ITS) sequence comparisons grouped all isolates within the *C. gloeosporioides* species complex, and the diverse morphology of the colonies and conidia agreed with this placement. These data suggest the fungicide failures reported by growers in central Florida were due to selection of QoI fungicide insensitivity in stem-infecting populations of *C. gloeosporioides*. Furthermore, insensitive isolates from fruits and leaves indicate that the fungicide azoxystrobin may no longer be a viable management option for ripe rot and anthracnose leaf spot, either. Additional efforts are needed to determine the nature and distribution of the resistance, to develop alternative management options, and to reinforce good resistance management practices for Florida's blueberry industry.

**Fungicide sensitivity of *Monilinia vaccinii-corymbosi* in highbush blueberries in Michigan**

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Mummy berry in blueberries, caused by the fungus *Monilinia vaccinii-corymbosi (Mvc),* is usually controlled with fungicides, in particular sterol inhibitors which have been used for almost 20 years. Fungicide sensitivity of *Mvc* isolates was studied across Michigan. Isolates were collected from various field sites and grown in pure culture: 72 isolates were tested in poison agar assays using six concentrations of three commonly used fungicides in the sterol inhibitor class (fenbuconazole, metconazole, and prothioconazole). Commercial formulations of these products were used. Colony diameter was measured after an incubation of 14 days at 22 degrees C in the dark and compared to growth on non-amended PDA. EC50 values were calculated for all isolates using a four-parameter logistical curve fit in the computer program R. EC50 values ranged from 0.0016 to 0.0146 ppm for fenbuconazole, 0.0007 to 0.0104 ppm for metconazole, and 0.0021 to 0.0986 ppm for prothioconazole. A slight shift towards reduced fungicide sensitivity was detected in more intensively managed fields compared to baseline sites; however, highly resistant isolates were not detected. The ITS region of the ribosomal DNA of the isolates was sequenced to confirm their identity and assess species-level genetic diversity. All isolates were nearly identical. Allthough the risk of sterol inhibitor resistance in *Mvc* appears to be relatively low, fungicide resistance management is still imperative.

**Induced overexpression of the gene *MfCYP51* may reveal molecular mechanisms associated to tebuconazole resistance of *Monilinia fructicola* in Brazil**

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The aim of this study was to investigate the mechanism associated with *Monilinia fructicola* resistance to demethylation inhibitor fungicide (DMI) tebuconazole in Brazil. In this study 10 isolates (8 DMI-R and 2 DMI-S) with broad tebuconazole sensitivity (0,003 - >10 µg/ml) were used to quantify the expression levels of the genes *MfCYP51* and *MfABC1*, respectively encoding the target enzyme 14 α-demethylase and mediating the transport of substances across biological membrane. After the mycelial exposure to sub-lethal doses of tebuconazole, the $2^{-\Delta\Delta Ct}$ method revealed increased *MfCYP51* mRNA levels for 6 out of 7 DMI-R isolates (ranging from 1.06 to 9.08), while DMI-S revealed small expressions levels of 0.14 and 0.46. The induced expression of the *MfABC1* gene with tebuconazole on isolate mycelium not always increased the levels of mRNA. The Mona element which likely trigger the elevated expression of *MfCYP51* was not present on 58 DMI-R isolates from Brazil including those with increased expression, suggesting that other genetic change yet to be discovered may be triggering this mechanism. Full *MfCYP51* sequencing is currently underway. This is the first report on molecular mechanisms for DMI resistance identification for *M. fructicola* isolates from Brazil; providing an important advancement for risk assessment of DMI fungicides used on brown rot management.

**Profiling of Resistance to SDHI Fungicides in *Botrytis cinerea* from Strawberry Fields**

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From 2012-13 to 2014-15, a total of 2,570 *B. cinerea* isolates were collected from strawberry fields in the eastern United States and their sensitivity to the SDHI fungicides including boscalid, fluopyram, fluxapyroxad, and penthiopyrad was assessed. Assays were based on visual assessment of presence or absence of mycelial growth on media amended with discriminatory doses of fungicides in microtiter plates. Overall frequencies of isolates resistant to boscalid, fluopyram, fluxapyroxad and penthiopyrad increased over the three years to 30.0, 1.0, 5.5, and 7.4%, respectively. Four resistance patterns designated A, B, C or D were found. Pattern A isolates were resistant to boscalid with the allele H272R at locus *sdhB*; Pattern B isolates were resistant to boscalid and penthiopyrad with the allele H272R or H272Y at locus *sdhB*; Pattern C isolates were resistant to boscalid, fluxapyroxad, and penthiopyrad with the allele H272Y at locus *sdhB*; and pattern D isolates were resistant to boscalid, fluopyram, fluxapyroxad, and penthiopyrad with alleles P225F or N230I at locus *sdhB*. Isolates with alleles H272R, H272Y, N230I or P225F were sensitive to a new SDHIs, benzovindiflupyr, with mean $EC_{50}$ values less than 0.5 µg/ml for each genotype, suggesting that this fungicide may be useful for resistance management.

**Resistance of *Colletotrichum gloeosporioides* from strawberry to azoxystrobin and reduced-sensitivity to thiophanate-methyl**

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*Colletotrichum gloeosporioides* causes Colletotrichum crown rot (CCR) of strawberry in the southern United States. Symptoms start with wilting of the plants which consequently collapse. A discoloration can be observed when the crown is cut open. CCR is managed with the use of disease-free transplants and fungicide applications. Our objective was to evaluate the sensitivity of *C. gloeosporioides* to two common fungicides used to manage CCR in Florida strawberry fields. Sixty-three *C. gloeosporioides* isolated from strawberry crowns from 1995 to 2015 were evaluated in a conidial