

Semiannual Newsletter

OVERVIEW

Rot Madness: Fighting Fungal Foes with Fungicides.....2

- Validation of methods to identify fungicide resistance in *Colletotrichum fioriniae*
- New *Botrytis* species associated with blueberry blossom blight
- Identifying national, regional and local fungicide use patterns in blueberry production

Nationwide Resistance Monitoring in *Botrytis* and *Colletotrichum*...3

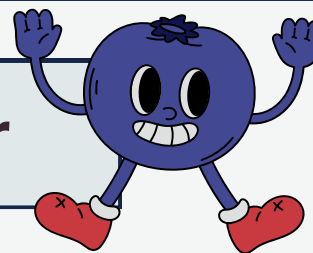
- Optimization of CRISPR/Cas9 and mutations found in *C. fioriniae* conferring fungicide resistance in 4 States
- 200 *B.cinerea* isolates recovered and a qPCR assay to detect multiple fungicide resistance mutations is validated

Timing Is Ripe: Fruit Maturity Drives Blueberry Susceptibility to Pathogens.....4

- Blueberry cultivar sensitivity to fungal infection varies by fruit maturity, not inoculation timing

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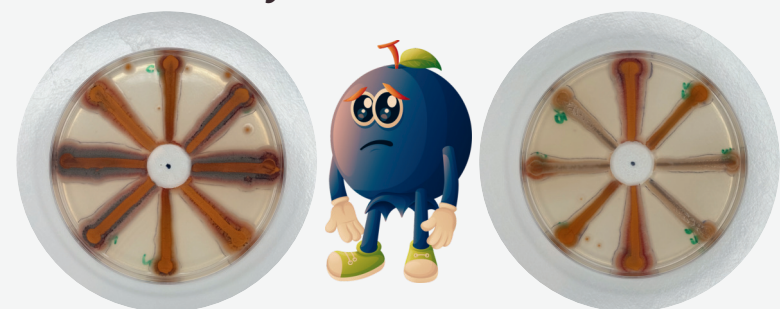
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ROT MADNESS: FIGHTING FUNGAL FOES WITH FUNGICIDES



New! Validation of methods to identify fungicide resistance in *Colletotrichum fioriniae* based on *cytb* mutations

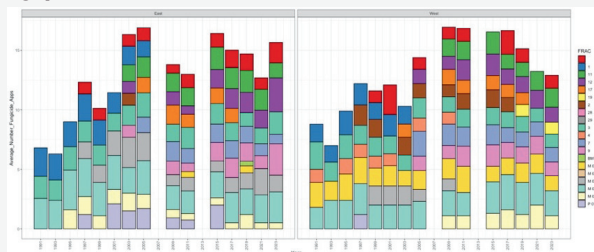


- The serial dilution growth assays, discriminatory dose assessment, and spiral gradient fungicide assay confirmed these mutations.
- These methods are useful for the efficient screening of extensive fungal collections (like ours! ~1000 isolates).



Alert! A new botrytis species causing blossom blight on blueberries in Michigan

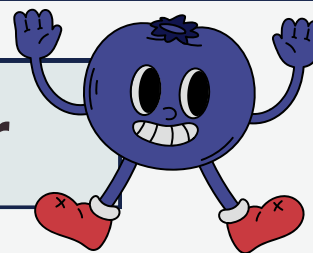
- The new species fall in a distinct clade closely related to *B. fabiopsis*, *B. caroliniana*, and *B. galanthina*.
- Pathogenicity tests confirmed its ability to cause disease, with severity comparable to *B. cinerea*.
- The new species were sensitive to all tested fungicides except cyprodinil.



Hot! Use of USDA NASS public data to identify national, regional and local fungicide use patterns in blueberry production

- Fungicide use diversified as new chemistries emerged; FRAC 19 and 4 were unused, and FRAC 2 and M 01 were minimally applied in the Eastern U.S.

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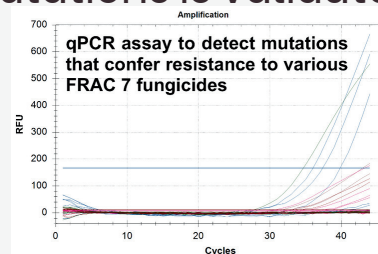
NATIONWIDE RESISTANCE MONITORING IN BOTRYTIS AND COLLETOTRICHUM



Jackie (Hu Lab), Mary and Aidan (Mattupalli Lab) (left to right) have been working on molecular techniques to identify fungicide resistance of *Botrytis* and *Colletotrichum* isolates nationwide

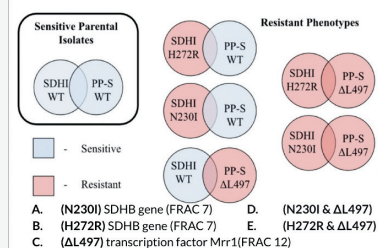
New! Optimization of CRISPR/Cas9 and mutations found in *C. fioriniae* conferring fungicide resistance in 4 States

Hot! 200 *B. cinerea* isolates recovered and a qPCR assay to detect multiple fungicide resistance mutations is validated

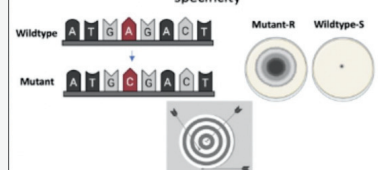


- At the Mattupalli Lab, Mary has been validating a qPCR assay to detect different fungicide resistance mutations in *B. cinerea* **all** at once.
- While Aidan collected 200 *B. cinerea* isolates from mature fruit samples from 17 fields in Washington to use through out the project.

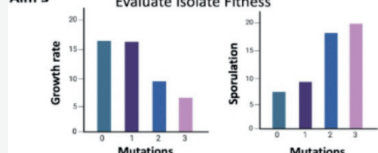
Aim 1 Gene-edited From Fungicide Sensitive to Resistant utilizing RNP based CRISPR/Cas9 via PEG mediated protoplast transformation



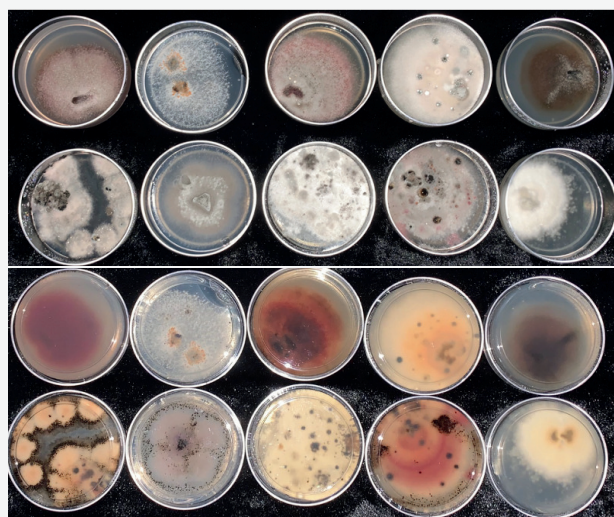
Aim 2 Confirm Genotypes, Phenotypes, and Editing specificity



Aim 3 Evaluate Isolate Fitness

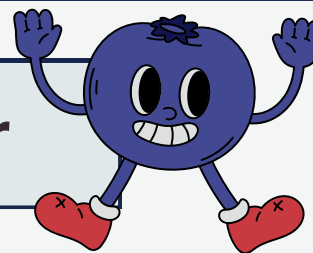


The Hu Lab has been confirming transformants and optimizing a CRISPR/Cas9 protocol for *Botrytis cinerea* through fulfilling Aim 1, 2 and 3.



- Sanger sequencing reveals *Colletotrichum* species and G143A mutation in *C. fioriniae* from four States.

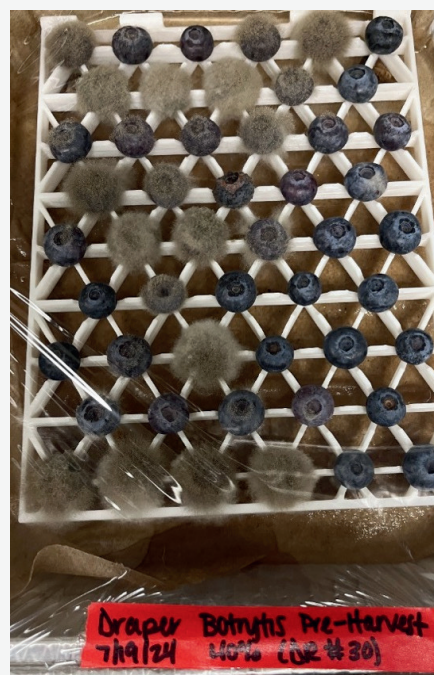
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TIMING IS RIPE: FRUIT MATURITY DRIVES BLUEBERRY SUSCEPTIBILITY TO PATHOGENS



Stephanie from the VanderWeide lab has been working on blueberry sensitivity to *B. cinerea* and *C. fioriniae* throughout fruit maturity stages



Hot! Blueberry cultivar sensitivity to fungal infection varies by fruit maturity, not inoculation timing

- Plants of three cultivars ('Duke', 'Draper', and 'Bluecrop') were inoculated with either *C. fioriniae* or *B. cinerea* during bloom or green fruit stages of development.
- We observed differences in the sensitivity of cultivars to both pathogens across berry maturity stages. However, these differences were less apparent across inoculation timing.
- We also designed 3D-printed trays to incubate blueberries following harvest.

