Possibilities for QoI resistance in North American populations of *Monilinia* species

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Outline

• *Monilinia* species attacking stone fruit in the Northeastern US
  – Species present and prevalence
  – Fungicide resistance in *Monilina* populations

• Cytochrome b gene
  – Background and relevance
  – Structure and practical implications

• Using the cyt b gene as a diagnostic tool
Monilinia species attacking stone fruit in the Northeastern US

- Prior to 2008: brown fruit rot by *Monilinia fructicola*
- 2008: shoot blight and brown fruit rot by *M. laxa*
  - 2008: Shoot blight in Niagara region
  - 2009: Brown fruit rot and shoot blight in Lake Ontario, Finger Lakes, & RI
  - Hosts: tart cherry, sweet cherry, ornamental cherry, & nectarine
- *M. laxa* likely always present, but never indentified
Monilinia species attacking stone fruit in the Northeastern US

Fungicide Resistance

- 2006-2009: Evaluated Monilinia populations from NY in regards to sensitivity to DMI and QoI fungicides
- DMI resistance and determinant ‘Mona’ widely present in NY M. fructicola populations
  - Most populations are from product failures
- ‘Mona’ not present in M. laxa populations and M. laxa isolates typically more sensitive to DMIs and QoIs even on the same tree
- Quantitative (phase) shifts toward QoI resistance in orchards with control failures
  - No indications of qualitative resistance
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Cytochrome b gene

- Mitochondrial gene encoding the target site enzyme complex for QoI fungicides
- Point mutations (G143A, F129L and G137R) in the cyt b gene ➔ qualitative resistance in fungal pathosystems
- Cloned full length coding and gene sequences from *M. fructicola*, *M. laxa*, and *M. frutigena*
- Examined 9 *M. fructicola*, 6 *M. laxa*, and 7 *M. frutigena* isolates for the presence of point mutations in the cyt b gene
  - Isolates from collections that represent the scope of QoI sensitivity and exposure for the region
**Cytochrome b gene**

<table>
<thead>
<tr>
<th>Species</th>
<th>Isolate</th>
<th>Relative growth (%)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>Resistance Status&lt;sup&gt;b&lt;/sup&gt;</th>
<th>Origin&lt;sup&gt;c&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>M. fructicola</em></td>
<td>MBH13B</td>
<td>25.6 ± 0.2</td>
<td>Baseline</td>
<td>Sweet cherry ‘Black Gold’/ Wayne County, NY</td>
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<td>MBH24A</td>
<td>22.4 ± 0.1</td>
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<td>MBH3B</td>
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<td>MBH12B</td>
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<td>ChocMF17</td>
<td>40.9 ± 0.1</td>
<td>Resistant</td>
<td>Peach ‘PF 25’/ Lancaster County, PA</td>
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<td>ChocMF64</td>
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<td>ChocMF63</td>
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<td>BltEBR08-1-1</td>
<td>36.9 ± 0.1</td>
<td>Resistant</td>
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<td>Peach7c</td>
<td>22.7 ± 0.3</td>
<td>Baseline</td>
<td>Peach ‘Baby Gold #5’/ Ontario County, NY</td>
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<td><em>M. laxa</em></td>
<td>EBRBa11b</td>
<td>14.4 ± 0.2</td>
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<td>Tart cherry ‘Montmorency’/ Niagara County, NY</td>
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<td>MbhMF08-14B</td>
<td>9.7 ± 0.3</td>
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<td>TLR53</td>
<td>0</td>
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<td>B.Sch#1</td>
<td>12.3 ± 0.1</td>
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<td>B.Sch#2</td>
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<td>B.Sch#4</td>
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<td><em>M. fructigena</em></td>
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<td>Mfg5-SP-A</td>
<td>N/A</td>
<td>N/A</td>
<td>Apple/Hungary</td>
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</tbody>
</table>

<sup>a</sup> Sensitivity percent colony growth on medium amended with analytical grade pyraclostrobin (1.0 µg mL<sup>-1</sup>) relative to that on non-amended medium.

<sup>b</sup> Practical resistance status ‘Baseline’ indicates no history of exposure to fungicides. ‘Sensitive’ (% relative growth <30) and ‘Resistant’ (% relative growth >30 and an orchard QoI control failure).
Cytochrome b gene

Botryotinia fuckeliana

Monilinia fructicola

Monilinia laxa

Monilinia fructigena
Cytochrome b gene

- *M. fructicola, M. laxa* and *B. fuckeliana* possess a group I-like intron immediately following codon 143
  - Development of the G143A mutation interfere intron removal during transcription → mutant isolate fails to make a functional cyt b gene → dies off

- *M. fructigena* lacks this same intron, suggesting that it may be at higher risk for developing the G143A mutation and associated QoI resistance

- None of the isolates had G143A, F129L or G137R point mutations or any other coding frame mutations
Using the *cytochrome b* gene as a diagnostic tool

- Primers were designed to a section of the cyt b allowing discrimination between the three species in a single PCR reaction: 621 bp (*M. fructicola*), 501 bp (*M. laxa*) and 783 bp (*M. fructigena*) fragments.

- Primers were tested against other fungi commonly associated with tree fruit orchards.

- Amplification was also attempted directly from infected fruit.
Using the *Cytochrome b* gene as a diagnostic tool
Summary

• Both *M. fructicola* and *M. laxa* are widely prevalent in NY, but if *M. fructigena* is/becomes present, we could detect it.

• *Monilinia* populations are only expressing quantitative resistance to QoIs.

• The group I-like intron may prevent the development of qualitative resistance to QoIs in *M. fructicola* & *M. laxa* populations.

• NY populations may develop a high level of quantitative resistance, but this could be tempered by high rates and fungicide rotation.
Acknowledgements

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Questions