Possibilities for Qol 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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- 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 Qol sensitivity and exposure for the region

			Resistance	
Species	Isolate	Relative growth (%) ^a	Status ^b	Origin ^c
M. fructicola	MBH13B	25.6 ± 0.2	Baseline	Sweet cherry 'Black Gold'/ Wayne County, NY
	MBH24A	22.4 ± 0.1	Baseline	Sweet cherry 'Black Gold'/ Wayne County, NY
	МВНЗВ	26.6 ± 0.2	Baseline	Sweet cherry 'Black Gold'/ Wayne County, NY
	MBH12B	16.8 ± 0.1	Baseline	Sweet cherry 'Black Gold'/ Wayne County, NY
	ChocMF17	40.9 ± 0.1	Resistant	Peach 'PF 25'/ Lancaster County, PA
	ChocMF56	44.7 ± 0.0	Resistant	Peach 'PF 25'/ Lancaster County, PA
	ChocMF63	42.6 ± 0.3	Resistant	Peach 'PF 25'/ Lancaster County, PA
	BitEBR08-1-1	36.9 ± 0.1	Resistant	Tart cherry 'Surefire'/ Niagara County, NY
	Peach7c	22.7 ± 0.3	Baseline	Peach 'Baby Gold #5'/ Ontario County, NY
M. laxa	EBRBa11b	14.4 ± 0.2	Sensitive	Tart cherry 'Montmorency'/ Niagara County, NY
	MbhMF08-14B	9.7 ± 0.3	Baseline	Sweet cherry 'Black Gold'/ Wayne County, NY
	TLRS3	0	Baseline	Sweet cherry 'Hedelfingen'l Ontario County, NY
	B.Sch#1	12.3 ± 0.1	Sensitive	Tart cherry ' Balaton'/ Niagara County, NY
	B.Sch#2	9.3 ± 0.2	Sensitive	Tart cherry ' Balaton'/ Niagara County, NY
	B.Sch#4	8.5 ± 0.1	Sensitive	Tart cherry ' Balaton'/ Niagara County, NY
M. fructigena	ES41	N/A	N/A	Apple/Spain
	ES49	N/A	N/A	Plum/Spain
	apple7	N/A	N/A	Apple/England
	apple13	N/A	N/A	Apple/England
	apple15	N/A	N/A	Apple/England
	Mfg4-GY-A	N/A	N/A	Apple/Hungary
	Mfg5-SP-A	N/A	N/A	Apple/Hungary

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

^b 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).



- *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 associated w
- Amplification 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 Qols
- 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

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Questions

