Detection of the G143A mutation in New York populations of *Podosphaera leucotricha* and its significance in QoI practical resistance

J.A. Hoffman[1,3], D.N. Henry[2,3], S.M. Villani[3], and K.D. Cox[3]

[1]Lycoming College, Department of Biology
[2]University of Kentucky, Department of Agriculture
[3]Cornell University, NYSAES, Department of Plant Pathology and Plant-Microbe Biology

Introduction

Apple powdery mildew, caused by the ascomycete *Podosphaera leucotricha*, is becoming increasingly prevalent in New York and New England apple production regions. The pathogen blights leaves, reduces bud set, and may cause fruit russeting, which results in an unmarketable crop. Site specific fungicides, such as quinone outside inhibitors (QoIs), have historically been effective in controlling powdery mildew infection while minimizing harmful effects to the environment[1]. QoI fungicides inhibit cellular respiration, and therefore ATP synthesis, by binding to the mitochondrial cytochrome *b* complex[1]. Unfortunately, this high level of specificity often favors the development of resistant pathogen populations, which has been the case in populations of *Venturia inaequalis,* the causal agent of apple scab[2]. In the northeastern United States, growers traditionally manage both pathogens concurrently using fungicides at similar timings. In *V. inaequalis,* a single point mutation within the cytochrome b (*cyt b*) gene has led to the development of QoI qualitative resistance[2]. This point mutation, G143A, results in a change from glycine to alanine at amino acid position 143 in the *cyt b* gene. Although the number of orchards with QoI resistant *V. inaequalis* populations has been increasing since 2007, resistance to this fungicide class in *P. leucotricha* populations has yet to be observed. Recently, NY growers have been reporting potential losses in efficacy in QoI fungicides controlling powdery mildew. Therefore, we endeavored to investigate the prevalence and consequence of the G143A mutation in NY populations of *P. leucotricha*.

Objectives

- Use PCR-RFLP analysis to determine the prevalence of the G143A mutation in Western NY populations of *P. leucotricha*
- Evaluate the efficacy of the QoI fungicide trifloxystrobin (Flint 50WG) on powdery mildew in a research orchard with a QoI resistant *V. inaequalis* population
- Quantify the relative abundance of the *P. leucotricha cyt b* resistant allele (A143) using allele-specific quantitative PCR and evaluate the significance of the mutation on QoI practical resistance

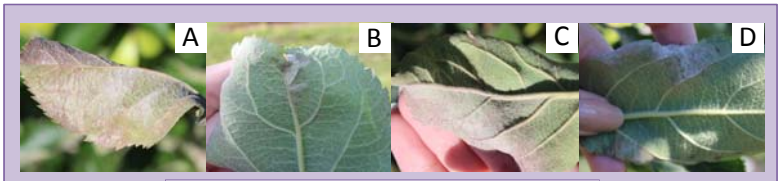

Figure 1. Symptoms of secondary apple powdery mildew development on the underside of "Jonagold' apple leaves. Trees were treated with **A)** No fungicide ('untreated') **B)** Topguard, a DMI fungicide; **C)** Captan 80WDG, a phthalimide fungicide; **D)** Flint 50WG, a QoI fungicide

Methods

PCR-RFLP Analysis

- Twenty-two composite samples of 25 individual leaf lesions were collected from eight orchards
- Primary or secondary mildew lesions were excised from apple leaves using a sterile cork borer
- Leaf tissue was ground in liquid nitrogen and DNA was extracted using an Omega BioTek DNA kit
- PCR-RFLP analysis was performed on the *cyt b* gene with restriction enzyme (Fnu4H1); products were separated on a 2% agarose gel

Fungicide Efficacy Control of Powdery Mildew

- An fungicide efficacy trial was conducted with four fungicide treatments: Flint 50WG (QoI), Topguard (DMI), Captan 80WDG, and an untreated check
- Powdery mildew severity was evaluated in mid July 2013 following secondary mildew development on 'Jonagold' apple terminal shoot leaves
- Mildew severity was assessed for eight fully expanded leaves counting back from the distal end of the terminal shoot; eight terminal shoots were assessed on each of four replicate trees for each treatment
- Disease severity data was evaluated using analysis of variance with generalized linear mixed models for a randomized complete block design using the GLIMMIX procedure of SAS v9.3 (SAS Institute, Cary, NC)

Allele-Specific Quantitative PCR

- Primer sets were designed to detect dG143 + A143 alleles or only the A143 allele
- Allele specific qPCR performed using Bio-Rad CFX96 Touch™ Real-Time PCR Detection System (Bio-Rad Inc., Hercules, Ca), with a SYBR Green 1 fluorescent dye detection system (Bio-Rad Inc.)
- Abundance of the A143 allele relative to the abundance of both G143 + A143 alleles in each sample was determined from standard curves using a set of serial dilutions of DNA from a highly resistant isolate as a quantitative comparison standard

Results

PCR-RFLP Analysis

Table 1. PCR-RFLP analysis of the G143A mutation region of the *P. leucotricha* cytochrome b gene for 22 composite samples

Sample	Band	G143A Mutation	Sample	Band	G143A Mutation
Ithaca Cortland 1	Yes	Yes	New Rogue Leaf 4	Yes	Yes
Ithaca Cortland 2	Yes	Yes	New Rogue Leaf 5	Yes	Yes
PMDNA RSFlint 1A	Yes	Yes	Burnap Leaf 1	Yes	Yes
PMDNA RSCON 1A	Yes	Yes	Little Tree 1	Yes	Yes
PMDNA EUP 1A	Yes	Yes	Little Tree 2	Yes	Yes
Brown Berry Patch Leaf 1	Yes	Yes	Little Tree 3	Yes	Yes
Brown Berry Patch Mix 1	Yes	Yes	Little Tree 4	Yes	Yes
Brown Berry Patch Mix 2	No	N/A	Glen Koehler 1	Yes	Yes
New Rogue Leaf 1	No	N/A	Glen Koehler 2	Yes	Yes
New Rogue Leaf 2	Yes	Yes	Traver 1	Yes	No
New Rogue Leaf 3	Yes	Yes	Traver 2	Yes	No

- A 208-bp amplicon was produced for 20 of 22 composite samples
- The G143A mutation was present in 18 of 20 isolates as verified by Fnu4H1 digestion
- Samples from Traver orchard were not digested by Fnu4H1 and were the only isolates without the G143A mutation

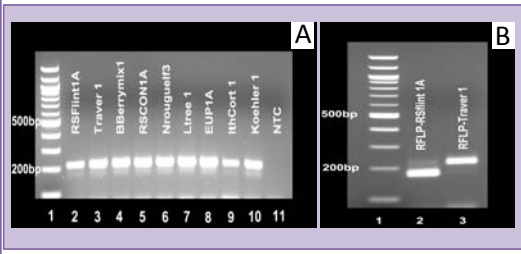

Figure 2. A) Gel electrophoresis of a 208-bp fragment in the G143A mutation region of the *P. leucotricha cyt b* gene. Lane 1: *100-bp DNA ladder*; lanes 2-10: *P. leucotricha* composite samples; lane 11: non-template control **B)** Gel electrophoresis following restriction digest with restriction enzyme Fnu4H1. Lane 2: QoI-resistant *P. leucotricha* sample RSFlint 1A; lane 3: QoI-sensitive *P. leucotricha* sample Traver 1

Fungicide Efficacy Control of Powdery Mildew

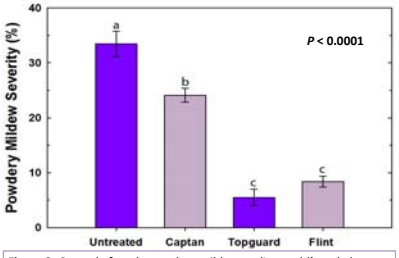

Figure 3. Control of apple powdery mildew on 'Jonagold' apple leaves, evaluated as a percentage of severity, in a Geneva, NY research orchard

- Powdery mildew percent severity ranged from 5.5% (Topguard) to 33.4% (untreated check)
- Topguard (DMI) and Flint 50WG (QoI) were most effective for powdery mildew control
- Captan 80WDG was less effective in controlling powdery mildew than the other fungicides
- *P. leucotricha* population from the research orchard in Geneva NY does not appear to have practical resistance to QoI fungicides

Allele-Specific Quantitative PCR

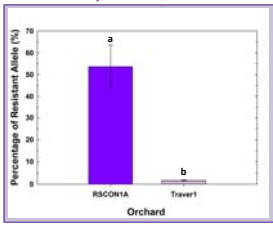

Figure 4. Resistant allele frequencies from *P. leucotricha* samples collected from 2 orchards, RSCON1A and Traver 1. RSCON1A was collected from the research orchard in Obj. 2

- Primers specific to the resistant allele successfully amplified a 196-bp fragment
- Frequency of the resistant allele was significantly higher for the RSCON1A sample compared to Traver 1 sample ($P < 0.0001$).

Conclusions

- Based on the presence of the G143A mutation in composite samples, qualitative resistance to QoI fungicides was present in the majority of orchards surveyed
- Despite the fact that the majority of the *P. leucotricha* population in the 'Jonagold' research orchard in Geneva, NY have the G143A mutation, practical resistance to the QoI fungicide trifloxystrobin (Flint 50WG) was not observed
- Compared to *V. inaequalis,* where only 20% of the G143A resistant allele is required to confer practical resistance[2], powdery mildew continues to be effectively controlled by Flint 50WG (trifloxystrobin) even though more than 50% of the resistant allele is present in the *P. leucotricha* population
- Evaluation of remaining *P. leucotricha* samples using allele-specific qPCR is ongoing

References

[1]Lesemann, S.S., et al (2006) Mitochondiral heteroplasmy for the cytochrome *b* gene controls the level of strobilurin resistance in the apple powdery mildew fungus *Podospora leucotricha. Journal of Plant Diseases and Protection*. 113(6): 259-266.

[2]Villani, S. M. and Cox, K. D. 2013. Quantification of heteroplasmy in the cytochrome b gene in *Venturia inaequalis* and its contribution to trifloxystrobin resistance. Phytopathology. XX:XX-X

Acknowledgements

Special thanks to Jess Raes, Kate Villani, Pippa Lehman, Jake Grimaldi, and Charlotte Lehman for their assistance throughout the project.