Within-season distribution of myclobutanil resistance in populations of *Venturia inaequalis* in Virginia

Sasha C. Marine[1], David G. Schmale III[1], and Keith S. Yoder[2]*

[1]Department of Plant Pathology, Physiology, and Weed Science, Virginia Tech, Blacksburg, Virginia 24061

[2]Virginia Tech AREC, Winchester, Virginia 22602

*Corresponding author: Professor of Plant Pathology, 595 Laurel Grove Road, Winchester, Virginia 22602 PH: (540) 869-2560, Email: ksyoder@vt.edu

ABSTRACT

Apple scab, caused by *Venturia inaequalis* (*Vi*), is a devastating disease of apple worldwide. Myclobutanil, a sterol-inhibiting (SI) fungicide, is commonly used to control apple scab in the eastern U.S. Populations of *Vi* in commercial apple orchards in Virginia have demonstrated resistance to myclobutanil and other SI fungicides, yet little is known about the nature and distribution of fungicide resistance in these populations. The objective of this study was to examine the within-season distribution of myclobutanil resistance in populations of *Vi* in Virginia. Shoot tips were banded on several branches of apple trees representing four cultivars in four different research blocks. Leaves with new apple scab lesions were collected in May, June, July and August of 2007, and over 400 isolates of *Vi* were cultured from these lesions. Fungicide resistance was evaluated in 82 of these isolates by monitoring their growth on agar plates containing 0 ppm, 0.1 ppm, 0.5 ppm, or 1 ppm myclobutanil (Nova 40W). Radial colony growth was measured weekly for 4 weeks. A similar range of fungicide resistance was observed for isolates collected at each sampling interval. After 4 weeks, agar treatments were significantly different ($P < 0.01$), but we did not observe a difference in fungicide resistance between isolates collected from treated or non-treated trees ($P = 0.63$). Sensitive and moderately resistant isolates were collected from both treated and non-treated trees. Repeated treatments of myclobutanil to a mixed population of *Vi* may result in a range of fungicide resistance in these populations, but this selective pressure may act over multiple growing seasons.

INTRODUCTION

- Apple scab, caused by *Venturia inaequalis*, causes unsightly lesions on apples, which significantly reduces their market value [1].
- Myclobutanil, a sterol-inhibiting fungicide, is often used to control apple scab [2]. The primary asset of this fungicide is that it can be used for after-infection control.
- Recent studies indicate myclobutanil and other SI fungicides are losing their effectiveness in controlling apple scab in Virginia [3,4].
- We hypothesized that populations of *Vi* from trees treated with myclobutanil increase in fungicide resistance throughout the growing season. The objective of this study was to examine the temporal dynamics of fungicide resistance in populations of *Vi* in Virginia.

MATERIALS AND METHODS

- Following infection periods, shoot tips were banded on several branches of treated and non-treated trees (FIG. 1). Myclobutanil (Nova 40W) was applied to treated trees at a rate of 1.25 oz/100 gal dilute, applied to runoff.
- Recent apple scab lesions were collected from four different research blocks at the Winchester AREC in 2007 (FIG. 2).
- Equal size agar plugs from the periphery of each isolate were transferred onto agar plates that contained 0 ppm, 0.1 ppm, 0.5 ppm, or 1 ppm myclobutanil.
- Radial growth was measured weekly for 4 weeks, and the entire experiment was repeated three times.
- Isolates were classified by their percent growth suppression based on the difference between mean colony growth on media amended with 0 ppm myclobutanil and 1 ppm myclobutanil at 28 days.
- ANOVA was used to test for significant differences among fungicide treatments, isolates collected from treated and non-treated trees, and isolate collection date. Data were analyzed using PROC GLM in SAS.

RESULTS AND DISCUSSION

1. After 4 weeks, agar treatments were significantly different ($P < 0.01$). In contrast, mean fungicide resistance level was not significantly different between treated or non-treated trees ($P = 0.63$) (Table 1).
2. There was no difference in fungicide resistance between isolates collected on May 24 and June 13, and on July 11 and August 14 (Table 2).
3. Sensitive and moderately resistant isolates were collected from both treated and non-treated trees.
4. A similar range of fungicide resistance was observed for isolates collected from treated (FIG. 3) and non-treated trees collected at each sampling interval.
5. Selective pressure towards fungicide resistance may act over multiple growing seasons.
6. Future research will continue to explore the temporal dynamics of fungicide resistance in *Vi* populations in Virginia.

REFERENCES

1. MacHardy, W.E. 1996. Apple Scab: Biology, Epidemiology, and Management. APS Press: St. Paul, MN.
2. Wilcox, W.F., Wasson, D.I, and Kovach, J. 1992. Development and evaluation of an integrated, reduced-spray program using sterol demethylation inhibitor fungicides for control of primary apple scab. Plant Dis. 76: 669-677.
3. Yoder, K. 1996. Orchard and laboratory testing of apple scab sensitivity to sterol-inhibiting fungicides. Virginia Fruit 1(41): 25-30.
4. Marine, S.C., Schmale III, D.G., and Yoder, K.S. 2007. Resistance to myclobutanil in populations of *Venturia inaequalis* in Winchester, Virginia. Plant Health Progress DOI:10.1094/PHP-2007-1113-01-RS.

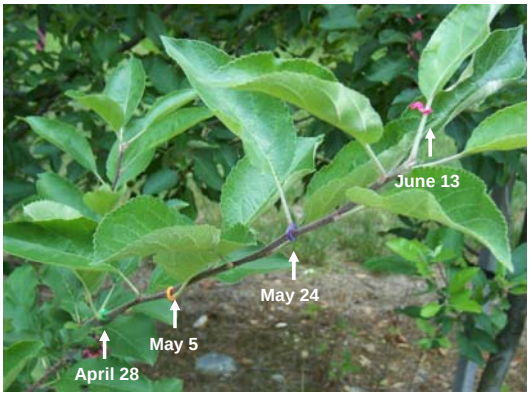

FIG. 1. Example of a banded shoot tip. Shoot tips were banded following an infection period with a different neon-colored rubberband.

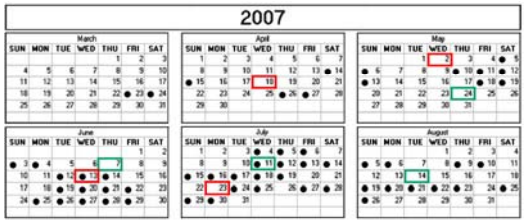

FIG. 2. Growing season in 2007. Collection dates are in green, fungicide treatment dates are in red, and apple scab infection periods are in black circles.

Factor	Type III SS, *P*-value for Different Assay Times			
	7 Days	14 Days	21 Days	28 Days
Cultivar	0.56	0.21	0.03	<0.01
Treatment	<0.01	<0.01	<0.01	<0.01
Treated or Non-Treated Tree	0.84	0.34	0.19	0.63
Collection Date	<0.01	<0.01	0.09	<0.01

Table 1. ANOVA for differences between factors when accounting for assay times.

Pairwise Comparison	7 Days	14 Days	21 Days	28 Days
May 24 x June 13	no difference	no difference	no difference	no difference
May 24 x July 11	difference	difference	no difference	difference
May 24 x August 14	difference	difference	no difference	difference
June 13 x July 11	difference	difference	no difference	difference
June 13 x August 14	difference	difference	no difference	difference
July 11 x August 11	no difference	no difference	no difference	no difference

Table 2. Differences in fungicide resistance between collection dates.

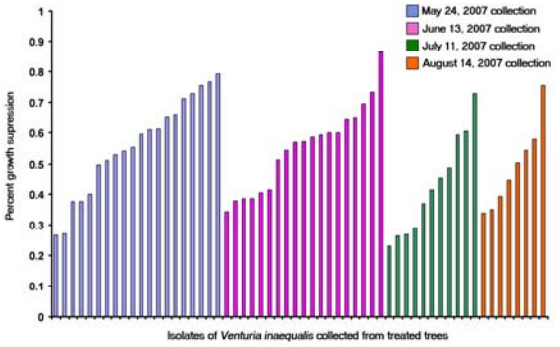

FIG. 3. Percent growth suppression of 58 isolates of *Venturia inaequalis* collected from trees treated with myclobutanil.