Prevalence of Myclobutanil Resistance and Difenconazole Insensitivity in Populations of *Venturia inaequalis*

Sara M. Villani, Section of Plant Pathology and Plant-Microbe Biology, School of Integrative Plant Science, Cornell University, Geneva, NY 14456; Alan R. Biggs, Kearneysville Tree Fruit Research and Education Center, West Virginia University, Kearneysville 25443; Daniel R. Cooley, Department Plant, Soil, and Insect Science, University of Massachusetts, Amherst 01003; and Jessica J. Raes and Kerik D. Cox, Section of Plant Pathology and Plant-Microbe Biology, School of Integrative Plant Science, Cornell University

Abstract

Villani, S. M., Biggs, A. R., Cooley, D. R., Raes, J. J., and Cox, K. D. 2015. Prevalence of myclobutanil resistance and difenconazole insensitivity in populations of *Venturia inaequalis*. Plant Dis. 99:1526-1536.

Demethylation inhibitors (DMIs) are a class of single-site fungicides with high levels of protective and curative efficacy against *Venturia inaequalis*, the causal agent of apple scab. To determine the prevalence of resistance to the DMI fungicide myclobutanil, 3,987 single-lesion conidial *V. inaequalis* isolates from 141 commercial, research, and baseline orchard populations were examined throughout New England, the mid-Atlantic, and the Midwest from 2004 to 2013. Of these orchard populations, 63% had practical resistance, 13% had reduced sensitivity, and 24% were sensitive to myclobutanil. A sensitivity baseline for the recently introduced DMI fungicide difenconazole was established to make comparisons with myclobutanil sensitivity in orchard populations. The mean effective concentration of difenconazole at which mycelial growth was inhibited

by 50% (EC₅₀) was determined to be 0.002 µg ml⁻¹ for 44 baseline isolates of *V. inaequalis*. From 2010 to 2013, 1,012 isolates of *V. inaequalis* from 37 of the 141 orchard populations above were screened for sensitivity to difenconazole. In all, 1 orchard population had reduced sensitivity to difenconazole, while the remaining 36 orchard populations were sensitive to the fungicide. In field experiments, difenconazole demonstrated high levels of apple scab control on mature apple fruit, despite the fact that the population of *V. inaequalis* had practical resistance to difenconazole. Although our results indicate widespread resistance to myclobutanil but not difenconazole, due to the propensity for cross-sensitivity among DMI fungicides, growers with myclobutanil resistance should be cautious when using difenconazole for disease management.

Apple scab, caused by the fungal pathogen *Venturia inaequalis* (Cooke) G. Winter, is among the most prevalent and economically important diseases in commercial apple orchards in regions with temperate climates worldwide (MacHardy 1996; MacHardy et al. 2001). The absence of durable apple scab resistance in the majority of commercially desired cultivars often mandates more than 10 fungicide applications each season to successfully manage the disease (Köller and Wilcox 2001; Lesniak et al. 2011; MacHardy 1996). Highly effective single-site fungicides such as the guanidines (1950s), sterile demethylation inhibitors (DMIs) (1980s), and quinone outside inhibitors (QoIs) (1990s) have enhanced our ability to manage a number of phytopathogenic fungi, including *V. inaequalis*. Of these fungicides, the DMIs have been especially attractive to growers because of their strong pre- and postinfection activity. Even 10 years after their introduction for use in commercial apple orchards in the United States, DMI fungicides had curative activity up to 96 h following an infection event (Beckerman et al. 2015).

DMI fungicides are a class of broad-spectrum, systemic compounds with high levels of activity against several plant-pathogenic fungi, including those that cause apple scab, rusts, and powdery mildews (Schnabel and Jones 2001). Historically, the DMI were most effective when used following infection events (Köller and Wilcox 1999; O'Leary et al. 1987; Schwabe et al. 1984; Szkolnik 1981), because they arrest fungal growth during and after infection by inhibiting demethylation at the 14- α carbon of lanosterol, a sterol intermediate of ergosterol, which is essential for hyphal growth. Although DMI fungicides are considered a medium risk for resistance by the fungicide resistance action committee (Stenzel 2014), the highly specific nature and repetitive use of DMI fungicides has led

to their diminished efficacy and widespread resistance in key apple production regions in North America (Braun and McRae 1992; Hildebrand et al. 1988; Jobin and Carisse 2007; Köller et al. 1997; Villani and Cox 2011).

In the history of apple scab management in the United States, the loss of one fungicide class due to resistance concerns has led to heavy reliance on the most recently registered alternative single-site fungicide class. For example, resistance to dodine by the 1970s was directly followed by dependence on and subsequent rapid onset of resistance to benzimidazoles (Jones 1981). Subsequently, the DMI fungicides and the more recently registered QoI fungicides became the industry standard for both protective and postinfection management of apple scab. As early as 1985, roughly 10 years after DMI fungicides were first applied commercially, individual *V. inaequalis* isolates were found to have reduced sensitivity to eight DMI fungicides (Stanis and Jones 1985).

Practical resistance, defined as greatly diminished levels of disease control following proper fungicide application under field conditions (Köller 1991; Villani and Cox 2014), was first reported for DMI in populations of *V. inaequalis* collected from a research orchard in Nova Scotia (Hildebrand et al. 1988) and later in a commercial orchard in Michigan (Köller et al. 1997). Since the initial reports, DMI fungicide resistance in populations of *V. inaequalis* has been documented in Virginia (Marine et al. 2007), Pennsylvania (Pfeuffer and Ngugi 2012), and Indiana (Chapman et al. 2011). Although individual isolates of *V. inaequalis* collected from New York orchards have previously been found to be resistant to the DMI fungicide fenarimol (Köller and Wilcox 1999), practical resistance to more heavily relied upon DMI fungicides in populations of *V. inaequalis* from the state has not been documented.

Since 2004, the authors have received increasing reports of practical resistance to myclobutanil (Nova 40W/Rally 40WSP) and fenarimol (Rubigan/Vintage SC) from apple growers in the mid-Atlantic and Northeastern United States. The failure of myclobutanil and other DMIs to provide acceptable levels of apple scab control has forced growers to modify their disease management programs. With limited single-site fungicide alternatives, producers are increasingly

Corresponding author: K. D. Cox; E-mail: kdc33@cornell.edu

Accepted for publication 16 March 2015.

<http://dx.doi.org/10.1094/PDIS-01-15-0002-RE>
© 2015 The American Phytopathological Society

relying on multisite protectant fungicides (i.e., captan and mancozeb) to manage apple scab. Protectant fungicides must be applied more frequently, have little to no postinfection activity, and are relatively ineffective (in comparison with DMI) in controlling apple powdery mildew (Köller et al. 1997), which should be managed during the same time as apple scab in the northeastern United States. In 2009, several DMI fungicides marketed previously in Europe were registered for management of apple scab in North America. These fungicides included difenoconazole and fenbuconazole, which have been found to have higher in vitro intrinsic activity (Pfeuffer and Ngugi 2012) and greater efficacy in the field (Cox et al. 2013) against *V. inaequalis* compared with myclobutanil.

The early reports of DMI control failures in the mid-Atlantic and the northeastern U.S. apple production regions, accompanied by the advent of newly released DMI fungicides for managing apple scab, prompted us to conduct an investigation of DMI fungicide resistance in *V. inaequalis* populations. The specific objectives of our study were to (i) determine the extent of myclobutanil resistance in orchard populations of *V. inaequalis* across the mid-Atlantic, the Midwest, and the northeastern United States from 2004 to 2013; (ii) establish baseline in vitro sensitivities for the DMI fungicide difenoconazole in *V. inaequalis*; (iii) evaluate and compare in vitro difenoconazole sensitivity for populations of *V. inaequalis* with practical resistance or sensitivity to myclobutanil; and (iv) determine the effect of myclobutanil or difenoconazole applications in monitored populations of *V. inaequalis* with practical resistance or sensitivity to myclobutanil.

Materials and Methods

Collection of *V. inaequalis* isolates. From 2004 to 2013, isolates of *V. inaequalis* were collected from 141 individual apple orchards throughout the northeastern United States. The orchards examined represent the range of DMI fungicide use practices and nearly all of the major apple production operations in the region, which consisted of 14 research, 119 commercial, and 8 baseline orchards. Some orchard populations were tested for DMI sensitivity because the grower had reported unsatisfactory control of apple scab that resulted in the appearance of apple scab symptoms following application of a DMI fungicide. However, nearly half of the orchard populations evaluated for sensitivity to myclobutanil were collected prior to local concerns over DMI performance by using bait trees or leaving trees unsprayed. Baseline orchards were designated as such if the orchard had never been exposed to any single-site fungicide classes, including the anilinopyrimidines, benzimidazoles, DMI, guanidines, QoI, or succinate dehydrogenase inhibitors.

Isolates from each orchard were obtained by arbitrarily collecting 50 to 100 young, fully expanded leaves that contained isolated young, sporulating primary apple scab lesions throughout the orchard. From each leaf collection, 50 leaves were selected and one individual lesion representing a single-spore infection event (Köller et al. 2004; MacHardy and Gadoury 1989) from each of those leaves was phenotyped for sensitivity to the DMI fungicide myclobutanil using microscopy-aided mycelial relative growth (RG) assays, as previously described and validated (Frederick et al. 2014; Köller et al. 2004; Villani and Cox 2014). Briefly, the single-conidial lesion isolates (referred to as “isolates” throughout the remainder of the manuscript) were removed using a sterile cork borer (5 mm in diameter), placed in 1.2 ml of sterile distilled water, and shaken for 60 s to dislodge conidia from the lesion. The leaf discs were promptly removed and 100- μ l suspensions of 10^3 to 10^4 *V. inaequalis* conidia ml^{-1} were evenly distributed on potato dextrose agar (PDA; Difco Laboratories) amended with streptomycin sulfate (50 $\mu\text{g ml}^{-1}$), chloramphenicol (50 $\mu\text{g ml}^{-1}$), and analytical grade myclobutanil (Sigma Aldrich) dissolved in acetone (Sigma Aldrich) at a discriminatory dose of 0.1 $\mu\text{g ml}^{-1}$, as suggested by Köller et al. (1991, 1997). Isolates were incubated at 22°C for 6 days, and mycelial growth of five randomly selected microcolonies originating from single spores was determined by measuring microcolony diameter or germ-tube length (for spores with greatly inhibited growth) using a SPOT Idea digital camera (with the SPOT Imaging Basic software package; Diagnostic Instruments Inc.) attached to an Olympus SZX12 stereoscope (Olympus America Inc.).

The in vitro sensitivity response for each isolate in each of the 141 orchard populations was expressed as mean percent relative growth (%RG), as previously described (Frederick et al. 2014; Köller et al. 1991; Smith et al. 1991). Each orchard population was subsequently characterized as having or lacking practical resistance to myclobutanil by comparing the distribution of sensitivity responses (mean %RG) for each orchard population in question to standards in which practical resistance or sensitivity to myclobutanil had already been verified. The practical resistance status of both the standard resistant orchard and the standard sensitive orchard was determined directly by examining fungicide application practices for each standard orchard and assessing the development of apple scab symptoms following application of Rally 40WSP or Nova 40W (myclobutanil; Dow Agrosciences). In 2011, orchard 61 had 5.8% incidence of apple scab on mature ‘Jerseymac’ fruit at harvest following four applications of Rally 40WSP and was used as the sensitive standard population. In 2010, orchard 26 had 45.3% incidence of apple scab at harvest on mature ‘McIntosh’ fruit following four applications of Rally 40WSP and was used as the resistant standard population. For both the sensitive standard and the resistant standard orchard populations, 50 young, fully expanded leaves that contained isolated young, sporulating primary apple scab lesions were collected throughout the orchard and in vitro sensitivity to myclobutanil was determined as described above. Comparisons of isolate sensitivity distributions to the standard orchard populations were made using the nonparametric Kolmogorov-Smirnov (K-S) two sample test in SAS (version 9.3; SAS Institute).

Using a K-S two-sample test, the practical resistance classification was determined for each of the 141 orchards by comparing the distribution of isolate myclobutanil responses for each orchard population (the “test orchard”) to sensitivity distributions of both the resistant and sensitive standards (Frederick et al. 2014). Initially, the probability that the distribution of myclobutanil sensitivity responses for a given test orchard was different from the distribution of the resistant standard orchard ($P_T = R$) or different from the distribution in myclobutanil sensitivity of the sensitive standard orchard ($P_T = S$) was determined. The outcome of the two probability tests was then used to classify the orchard population in one of four scenarios. For instance, if a given test orchard had $P_T = R < 0.05$ and $P_T = S \geq 0.05$, the sensitivity distribution of the orchard in question would be significantly different from the resistant standard but not from the sensitive standard; thus, the test orchard would be classified as “sensitive”. Alternatively, if a given test orchard had $P_T = R \geq 0.05$ and $P_T = S < 0.05$, the sensitivity distribution of the orchard in question would be significantly different from the sensitive standard but not from the resistant standard and, thus, the test orchard would be classified as “resistant”. In cases where the sensitivity distribution for a test orchard was not significantly different from the standard resistant orchard and the standard sensitive orchard ($P_T = R \geq 0.05$ and $P_T = S \geq 0.05$), the population in the test orchard would be considered “reduced sensitive” to myclobutanil. Finally, the distribution of a test orchard population may be significantly different from the sensitive standard and the resistant standard. Such a situation may happen when the distribution of the RG of isolates in the test orchard population was significantly greater than the resistant standard orchard or when the distribution of the RG of isolates in the test orchard population was significantly lower than the sensitive standard orchard. In such situations, an orchard would be classified as “resistant” or “sensitive”, respectively. Although sensitivity classifications were based on the distribution of isolate sensitivities within a population of *V. inaequalis*, the mean %RG was reported for each orchard population to facilitate comparisons between orchards. Thus, in some instances, sensitivity classifications (i.e., resistant and reduced sensitive), which were determined using the K-S statistical analysis, will have overlapping mean %RG values. In all cases, it is important to understand that classification of a test orchard only implies how its population would behave in regards to that of the standards for the purpose of discussing risk of product failure and the prevalence of resistance to DMI. It is not meant to be used to emphatically indicate product failure regardless of host and environment.

Table 1. Sensitivity classification, percent relative growth, isolation year, and collection location from which *Venturia inaequalis* populations were surveyed for myclobutanil resistance

Orchard ^a	Classification ^b	<i>n</i>	%RG ^c	Pr > Ksa resistant ^d	Pr > Ksa sensitive ^d	Year	State
1	Resistant	30	131.6 ± 13.8	0.0020	<0.0001	2005	New York
3	Resistant	32	96.9 ± 5.9	0.0003	<0.0001	2009	New York
5	Resistant	31	94.5 ± 8.0	<0.0001	<0.0001	2005	New York
6	Resistant	27	58.4 ± 5.9	0.4125	0.0439	2007	Massachusetts
8	Resistant	30	67.7 ± 5.7	0.6984	0.0011	2007	New York
9	Resistant	30	111.8 ± 9.0	0.0004	<0.0001	2006	New York
12	Resistant	30	113.7 ± 11.0	0.0003	<0.0001	2007	New York
16	Resistant	30	87.4 ± 6.6	0.0714	<0.0001	2007	New Hampshire
18	Resistant	29	86.1 ± 4.7	0.0646	<0.0001	2006	Massachusetts
19	Resistant	26	96.4 ± 3.6	<0.0001	<0.0001	2013	Vermont
20	Resistant	30	95.8 ± 6.9	0.0016	<0.0001	2006	New York
21	Resistant	16	80.0 ± 7.0	0.1268	<0.0001	2007	Maine
24	Resistant	30	66.5 ± 3.9	0.6243	0.0006	2007	Maine
25	Resistant	28	65.3 ± 5.6	0.5924	0.0016	2006	Massachusetts
26	Resistant	36	67.7 ± 3.4	1.0000	0.0055	2010	New York
27	Resistant	22	61.0 ± 6.5	0.2757	0.0245	2006	Rhode Island
31	Resistant	30	85.0 ± 8.0	0.0456	0.0003	2007	New York
34	Resistant	19	75.1 ± 7.1	0.4401	0.0002	2007	New York
35	Resistant	31	75.0 ± 4.3	0.1869	<0.0001	2005	Ohio
37	Resistant	35	96.4 ± 2.2	<0.0001	<0.0001	2013	New York
38	Resistant	21	72.7 ± 7.5	0.3866	0.0029	2008	Rhode Island
39	Resistant	30	75.7 ± 6.7	0.3701	0.0008	2007	Rhode Island
42	Resistant	30	69.3 ± 5.1	0.0015	0.5806	2006	Massachusetts
43	Resistant	31	64.1 ± 5.8	0.069	0.002	2012	Maine
45	Resistant	30	66.0 ± 3.6	0.3415	<0.0001	2006	New York
47	Resistant	38	113.5 ± 13.1	0.0006	<0.0001	2008	Rhode Island
48	Resistant	47	89.7 ± 3.7	0.0010	<0.0001	2008	West Virginia
49	Resistant	30	83.7 ± 3.8	0.0308	<0.0001	2006	New York
53	Resistant	30	108.2 ± 11.3	0.0105	<0.0001	2007	West Virginia
54	Resistant	17	109.5 ± 8.8	0.0017	<0.0001	2008	New York
55	Resistant	30	84.0 ± 8.8	0.2610	0.0003	2006	Massachusetts
56	Resistant	30	79.6 ± 5.8	0.0701	<0.0001	2006	Massachusetts
57	Resistant	30	76.2 ± 4.9	0.2849	<0.0001	2007	New York
59	Resistant	30	94.8 ± 7.1	0.0078	<0.0001	2007	Connecticut
60	Resistant	30	77.8 ± 3.1	0.0701	<0.0001	2007	New York
63	Resistant	22	82.9 ± 5.0	0.0471	<0.0001	2007	New York
64	Resistant	30	92.4 ± 8.2	0.0123	<0.0001	2006	New York
67	Resistant	30	69.8 ± 6.4	0.4642	0.0010	2007	Massachusetts
68	Resistant	30	74.9 ± 5.1	0.1926	<0.0001	2005	New York
70	Resistant	30	73.7 ± 5.2	0.4746	0.0008	2006	West Virginia
72	Resistant	31	85.7 ± 4.0	0.0111	<0.0001	2005	Ohio
74	Resistant	30	66.4 ± 4.1	0.7860	0.0005	2005	New York
75	Resistant	30	80.1 ± 5.2	0.0625	0.0003	2007	Massachusetts
77	Resistant	31	82.2 ± 8.2	0.4104	<0.0001	2005	West Virginia
78	Resistant	30	97.0 ± 8.4	0.0048	<0.0001	2005	New York
80	Resistant	30	105.6 ± 5.9	0.0002	<0.0001	2005	New York
82	Resistant	30	73.9 ± 3.6	0.1363	<0.0001	2005	Ohio
83	Resistant	30	63.0 ± 4.7	0.4261	0.0050	2007	New York
84	Resistant	31	67.6 ± 6.7	0.3556	0.0050	2008	Rhode Island
87	Resistant	30	68.7 ± 5.0	0.6056	<0.0001	2005	Ohio
88	Resistant	37	87.8 ± 4.7	0.0008	<0.0001	2013	New York
90	Resistant	34	80.7 ± 4.8	0.0295	<0.0001	2009	Pennsylvania
94	Resistant	26	83.8 ± 13.3	0.8396	0.0015	2006	Massachusetts
96	Resistant	30	82.9 ± 4.8	0.0244	<0.0001	2005	West Virginia
97	Resistant	30	90.7 ± 3.7	0.0020	<0.0001	2007	Rhode Island
98	Resistant	25	84.6 ± 8.5	0.1736	0.0001	2008	New York
99	Resistant	35	94.2 ± 7.6	0.0105	<0.0001	2008	New York

(continued on next page)

^a Orchard designations in bold designate the resistant standard population and the sensitive standard population used for determining the myclobutanil sensitivity classification according to the Kolmogorov-Smirnov (K-S) two-sample test. Both the resistant and sensitive standard are designated in bold text.

^b Orchard classification was determined by comparing the distribution of myclobutanil sensitivity responses (isolate percent mean relative growth [%RG] values) for a given *V. inaequalis* orchard population with standard orchard populations with and without practical resistance to myclobutanil using the K-S two-sample test in SAS (version 9.3; SAS Institute, Cary, NC). Red-sens. = reduced sensitive.

^c Orchard population mean percent growth of *V. inaequalis* isolates on medium amended with analytical-grade myclobutanil at 0.1 µg ml⁻¹ relative to that on nonfungicide medium (%RG). Values are means and standard errors of *n* isolates for each population, with five single-conidium colonies for each isolate.

^d Probability that the distribution of sensitivity responses for the orchard population in question is not significantly different from the standard orchard population with (Pr > Ksa resistant) or without (Pr > Ksa sensitive) practical resistance to myclobutanil.

Table 1. (continued from preceding page)

Orchard ^a	Classification ^b	n	%RG ^c	Pr > Ksa resistant ^d	Pr > Ksa sensitive ^d	Year	State
100	Resistant	30	61.8 ± 4.4	0.0877	0.0010	2006	New York
102	Resistant	34	61.6 ± 5.0	0.9072	0.0097	2008	Massachusetts
103	Resistant	27	82.0 ± 11.1	0.0273	<0.0001	2008	Rhode Island
105	Resistant	32	114.8 ± 13.8	<0.0001	<0.0001	2005	Ohio
106	Resistant	30	65.9 ± 7.6	0.2317	0.0092	2007	New York
107	Resistant	33	69.1 ± 6.1	0.0885	0.0004	2009	New York
108	Resistant	41	53.1 ± 5.6	0.5169	0.0347	2009	Michigan
109	Resistant	30	73.6 ± 7.4	0.2973	0.0050	2007	New York
111	Resistant	30	79.4 ± 11.3	0.4754	<0.0001	2007	Rhode Island
113	Resistant	30	93.9 ± 8.1	0.1050	<0.0001	2007	New York
117	Resistant	30	78.7 ± 5.5	0.1387	<0.0001	2004	New York
118	Resistant	29	60.0 ± 4.5	0.3550	0.0040	2007	New York
120	Resistant	30	84.0 ± 6.9	0.0625	<0.0001	2005	West Virginia
121	Resistant	29	80.3 ± 7.9	0.4495	<0.0001	2006	Rhode Island
122	Resistant	30	64.4 ± 4.4	0.6984	0.0008	2005	New York
123	Resistant	30	99.1 ± 11.0	0.0053	<0.0001	2007	New York
124	Resistant	30	116.4 ± 11.1	<0.0001	<0.0001	2006	Massachusetts
125	Resistant	30	132.4 ± 12.7	<0.0001	<0.0001	2005	New York
127	Resistant	30	94.4 ± 4.3	0.0004	<0.0001	2005	Wisconsin
141	Resistant	21	73.4 ± 7.3	0.8552	0.0002	2010	Maine
148	Resistant	21	77.9 ± 2.3	0.0008	<0.0001	2011	New York
149	Resistant	21	94.9 ± 3.0	<0.0001	0.0004	2009	Massachusetts
150	Resistant	18	93.4 ± 5.6	0.0115	<0.0001	2011	Virginia
154	Resistant	16	98.4 ± 5.2	0.0033	<0.0001	2011	North Carolina
157	Resistant	31	82.0 ± 3.5	0.8639	<0.0001	2012	Vermont
158	Resistant	24	68.8 ± 4.5	0.2249	0.0015	2011	Maine
162	Resistant	24	60.3 ± 4.2	0.1778	0.0096	2012	Michigan
163	Resistant	36	78.0 ± 3.5	0.7264	<0.0001	2012	Maine
170	Resistant	33	76.3 ± 3.9	0.7767	<0.0001	2012	New York
172	Resistant	35	70.0 ± 2.6	0.7497	<0.0001	2012	New York
173	Resistant	17	87.2 ± 8.1	0.0448	0.0020	2013	Vermont
175	Resistant	26	99.2 ± 3.6	<0.0001	<0.0001	2013	New York
14	Red-sens.	32	49.9 ± 8.3	0.0868	0.1888	2009	Michigan
22	Red-sens.	30	51.3 ± 3.2	0.0055	0.0043	2005	Massachusetts
30	Red-sens.	24	55.8 ± 7.9	0.2423	0.3291	2007	Massachusetts
33	Red-sens.	30	50.2 ± 4.0	0.0055	0.0215	2007	New Hampshire
44	Red-sens.	30	60.6 ± 13.8	0.4261	0.0751	2007	Rhode Island
51	Red-sens.	30	57.5 ± 4.2	0.0429	0.0007	2006	New York
62	Red-sens.	30	59.5 ± 6.7	0.0877	0.0751	2006	New York
76	Red-sens.	30	54.3 ± 5.0	0.0484	0.0076	2006	Vermont
89	Red-sens.	30	49.2 ± 2.8	0.0063	0.0119	2005	New Hampshire
104	Red-sens.	24	48.0 ± 2.5	<0.0001	0.0068	2011	Maine
110	Red-sens.	34	55.3 ± 3.2	0.0001	0.0053	2012	Vermont
142	Red-sens.	35	48.3 ± 1.8	<0.0001	0.0183	2013	Michigan
147	Red-sens.	23	62.3 ± 3.8	0.0158	0.0004	2012	New York
153	Red-sens.	20	52.5 ± 3.4	<0.0001	0.0068	2011	Maine
164	Red-sens.	37	58.9 ± 3.4	0.0006	0.0027	2012	New York
165	Red-sens.	19	62.6 ± 3.4	0.0117	0.0014	2012	Massachusetts
171	Red-sens.	34	68.8 ± 3.3	0.024	<0.0001	2012	New York
174	Red-sens.	37	49.5 ± 3.3	0.0046	0.0158	2013	Maine
4	Sensitive	30	50.0 ± 6.7	0.0025	0.1600	2007	New Hampshire
11	Sensitive	30	50.8 ± 4.4	0.0356	0.0842	2007	Rhode Island
13	Sensitive	26	41.2 ± 13.8	<0.0001	0.2924	2011	New York
23	Sensitive	30	27.8 ± 2.1	<0.0001	0.0842	2007	New York
28	Sensitive	17	12.9 ± 3.4	<0.0001	0.0088	2010	New York
29	Sensitive	36	30.9 ± 2.1	<0.0001	0.1243	2008	Rhode Island
32	Sensitive	17	23.5 ± 7.5	0.0004	0.0494	2009	Michigan
41	Sensitive	30	37.5 ± 4.9	<0.0001	0.4260	2007	Rhode Island
46	Sensitive	22	13.7 ± 5.2	<0.0001	0.0004	2009	Michigan
50	Sensitive	33	36.4 ± 6.7	0.0023	0.1945	2009	New York
52	Sensitive	15	29.4 ± 6.9	0.0183	0.9048	2009	Ohio
58	Sensitive	20	29.3 ± 6.5	0.0017	0.3446	2009	Michigan
61	Sensitive	36	38.4 ± 3.6	0.0007	1.0000	2011	New York
69	Sensitive	19	21.9 ± 6.2	0.0009	0.1575	2005	New York
71	Sensitive	16	33.6 ± 2.9	0.0002	0.4930	2004	New York
73	Sensitive	30	43.9 ± 3.2	0.0006	0.0751	2006	West Virginia
85	Sensitive	27	23.8 ± 5.3	<0.0001	0.1573	2009	Rhode Island

(continued on next page)

Table 1. (continued from preceding page)

Orchard ^a	Classification ^b	n	%RG ^c	Pr > Ksa resistant ^d	Pr > Ksa sensitive ^d	Year	State
86	Sensitive	17	33.0 ± 6.6	0.0054	0.4576	2013	Maine
92	Sensitive	34	9.0 ± 2.1	<0.0001	0.0002	2009	Indiana
93	Sensitive	20	25.7 ± 9.3	<0.0001	0.0096	2009	Maine
101	Sensitive	22	30.0 ± 3.5	<0.0001	0.5401	2012	New York
112	Sensitive	30	54.7 ± 4.5	0.0066	0.1445	2006	New York
115	Sensitive	24	40.5 ± 5.5	0.0026	0.8186	2005	Massachusetts
116	Sensitive	33	46.2 ± 6.4	0.0109	0.7252	2009	New York
119	Sensitive	19	46.9 ± 12.2	0.0063	0.4413	2010	West Virginia
128	Sensitive	33	37.9 ± 2.8	0.0001	0.2321	2004	New York
151	Sensitive	24	42.6 ± 3.6	<0.0001	0.1725	2011	Maine
152	Sensitive	26	43.7 ± 3.1	<0.0001	0.0613	2011	Maine
155	Sensitive	15	51.1 ± 4.4	0.0002	0.0506	2011	Maine
161	Sensitive	15	47.4 ± 9.3	0.0015	0.5137	2010	New York
166	Sensitive	31	16.1 ± 1.7	<0.0001	0.0002	2012	Maine
167	Sensitive	33	36.7 ± 2.1	<0.0001	0.1618	2012	Maine
168	Sensitive	25	30.6 ± 4.9	<0.0001	0.5061	2012	Maine
169	Sensitive	24	51.6 ± 5.0	0.001	0.0621	2012	Maryland

Baseline sensitivity and sensitivity of populations of *V. inaequalis* to difenoconazole. In order to develop a baseline for difenoconazole sensitivity in *V. inaequalis*, 44 *V. inaequalis* single conidial lesion isolates were collected from two baseline apple orchards located in Geneva, NY, in 2010 and were screened for sensitivity to difenoconazole. For each single conidial lesion isolate, conidial suspensions were prepared as described above and were evenly distributed on PDA medium that was amended with streptomycin sulfate (50 µg ml⁻¹), chloramphenicol (50 µg ml⁻¹), and analytical grade difenoconazole (Sigma Aldrich) at final concentrations of 0.0, 0.0001, 0.0005, 0.001, 0.01, 0.05, 0.1, 0.5, and 1 µg ml⁻¹. Following 6 days of incubation at 22°C, mycelial growth of five randomly selected microcolonies originating from single spores was determined by measuring microcolony diameter or germ tube length (for spores with greatly inhibited growth) using a SPOT Idea digital camera attached to an Olympus SZX12 stereoscope, as described above. Relative percent inhibition was calculated at each fungicide concentration as previously described (Yoshimura et al. 2004). For each isolate, dose response curves were constructed using relative percent inhibition of colony growth at each log-transformed (log₁₀) concentration for the respective fungicide to determine the value of the effective concentration that inhibited isolate growth by 50% (EC₅₀).

From 2010 to 2013, single conidial lesion isolates that were used for the determination of myclobutanil sensitivity were also screened for sensitivity to analytical grade difenoconazole at a discriminatory concentration of 0.1 µg ml⁻¹. This concentration was chosen on the basis of being able to make comparisons of intrinsic activity with myclobutanil. Difenoconazole sensitivity, expressed as %RG, was determined in an identical manner as the myclobutanil sensitivity assay described above. Orchards were classified as sensitive, reduced sensitive, or resistant using the K-S two-sample test, as described above. Because control failures with difenoconazole have not been reported in the region, we expected that the sensitivity responses of *V. inaequalis* isolates to difenoconazole in vitro would have to approach the same level of growth response that allows an isolate or population to achieve resistance to myclobutanil in order for practical resistance to be observed. Thus, the DMI-sensitive and -resistant orchards used for the classification of myclobutanil sensitivity were used to classify orchard sensitivity to difenoconazole using the same sensitive and resistant thresholds.

Myclobutanil and difenoconazole cross-sensitivity in a representative baseline (orchard 28) and a myclobutanil-resistant population of *V. inaequalis* (orchard 37) was also evaluated in 2013. In all, 17 isolates from a baseline orchard and 35 isolates from a myclobutanil-resistant orchard were assayed for sensitivity to both fungicides. Simple linear regression analysis was used to determine whether there was a relationship between RG responses between the two fungicides in vitro (SigmaPlot version 11.0; Systat Software Inc.).

Impact of DMI fungicide applications on myclobutanil and difenoconazole sensitivity. From 2010 to 2013, sensitivity to myclobutanil and difenoconazole was evaluated in a research orchard composed of 15-year-old 'Empire' apple on M.9/M.111 rootstocks. Prior to 2010, myclobutanil had provided acceptable levels of control (i.e., <20% incidence) on mature fruit (Cox et al. 2009), and trees within this research orchard had only received a total of 12 applications of myclobutanil during its production history. To determine the effect of difenoconazole applications on *V. inaequalis* populations sensitive to DMI fungicides, four applications of Inspire (difenoconazole; Syngenta) were made each year from 2010 to 2013 at the maximum labeled rate of 293 ml ha⁻¹. Inspire was applied in approximately 2,800 liters of water per hectare using an AA2 GunJet handgun (TeeJet Technologies) at approximately 2,000 kPa pressure. Treatments were applied on approximately 7- to 10-day intervals at the phenological timings of pink, bloom, first cover, and second cover. Isolated primary apple scab lesions were collected in the spring of 2010 to 2013 and were assayed for sensitivity to myclobutanil and difenoconazole, as described above.

To determine the impact of DMI fungicide applications and the degree of stability of myclobutanil resistance, changes in myclobutanil sensitivity were evaluated in a 30-year-old McIntosh and 'Cortland' research apple orchard with a *V. inaequalis* population previously confirmed to have practical resistance to myclobutanil (Turechek et al. 2005). From 2005 to 2010, Nova 40W or Rally 40WSP was applied at a rate of 333 g ha⁻¹. Rally 40WSP was applied at the same phenological timings using the same application parameters as described above for the Empire planting. From 2011 to 2013, the myclobutanil selective pressure was removed from the orchard and DMI fungicides were no longer applied during the remainder of the study. In each year, isolated primary apple scab lesions were collected in the spring of 2005 to 2013 and were assayed for sensitivity to myclobutanil as described above.

Results

Myclobutanil sensitivity of *V. inaequalis* orchard populations. From 2004 to 2013, 141 individual populations of *V. inaequalis* throughout the northeastern United States were evaluated for sensitivity to myclobutanil (Table 1). In total, 3,987 single-conidial lesion isolates of *V. inaequalis* were tested for sensitivity to myclobutanil, with 107 of the orchard populations being represented by 25 or more isolates. The remaining 34 orchard populations were represented by a minimum of 15 isolates.

Throughout the 10-year study, 89 of the 141 (63%) orchard populations of *V. inaequalis* were determined to have practical resistance to myclobutanil on the basis of statistical comparisons with sensitive and resistant population standards. The mean %RG of the orchards with practical resistance ranged from 53.1 to 132.4%, with a research

orchard population from East Lansing, MI (orchard 108: $53.1 \pm 5.6\%$) and a commercial orchard population from Chazy, NY (orchard 125: $132.4 \pm 12.7\%$) having the lowest and highest RG, respectively. Seventy-nine of the orchard populations exhibiting practical resistance to myclobutanil were commercial orchards. Within these commercial orchards, myclobutanil (Nova 40W or Rally 40WSP) was applied for an average of 13 years with two to four applications of the fungicide per season. The remaining orchard populations classified as having practical resistance to myclobutanil were from research orchards in which DMI fungicides had been applied regularly throughout the planting history.

Only 18 (13%) orchard populations of *V. inaequalis* were found to have reduced sensitivity to myclobutanil. The mean %RG for these populations ranged from 48.0 to 68.8% with an orchard from Wells, ME (orchard 104: $48.0 \pm 2.5\%$) and an orchard from Sodus, NY (orchard 171: 68.8 ± 3.3) representing the minimum and maximum endpoints of the reduced-sensitive designation. Sixteen orchard populations with reduced sensitivity were from commercial orchards and, in these orchards, applications of myclobutanil were made for an average of 11 years with two to four applications per season. One research orchard (orchard 62) in which DMI fungicides had been applied regularly throughout the history of the orchard and one baseline orchard (orchard 51) that had reportedly never directly been exposed to fungicides were determined to have reduced sensitivity to myclobutanil.

Table 2. Summary of *Venturia inaequalis* orchard populations by region and state classified on the basis of in vitro sensitivity to the demethylation inhibitor fungicides myclobutanil and difenoconazole

Classification, region, state ^a	Orchard populations			
	Myclobutanil		Difenoconazole	
	<i>n</i>	For state (%)	<i>n</i>	For state (%)
Resistant				
New England				
Connecticut	1	100	...	...
Massachusetts	12	75	0	0
Maine	6	35	0	0
New Hampshire	1	25	...	...
Rhode Island	9	64	...	...
Vermont	3	60	0	0
Mid-Atlantic				
Maryland	0	0	0	0
North Carolina	1	100	...	...
New York	40	70	0	0
Pennsylvania	1	100	...	...
Virginia	1	100	0	0
West Virginia	6	75	0	0
Midwest				
Indiana	0	0	...	...
Ohio	5	83	...	...
Michigan	2	29	0	0
Wisconsin	1	100	...	...
Total	89	63	0	0
Reduced sensitive				
New England				
Connecticut	0	0	...	...
Massachusetts	3	19	0	0
Maine	3	18	0	0
New Hampshire	2	50	...	...
Rhode Island	1	7	...	...
Vermont	2	40	0	0

(continued in next column)

^a Orchard practical resistance classification was determined by comparing the distribution of myclobutanil or difenoconazole sensitivity responses (isolate percent mean relative growth values) for a given *V. inaequalis* orchard population with standard orchard populations with and without practical resistance to myclobutanil using the Kolmogorov-Smirnov two-sample test in SAS (version 9.3; SAS Institute, Cary, NC).

Thirty-four orchard populations of *V. inaequalis* (24%) were found to be sensitive to myclobutanil. The mean %RG of sensitive populations was 9.0 to 54.7%, with a baseline population in West Lafayette, IN (orchard 9: 9.0 ± 2.1) and a commercial orchard surveyed in 2006 from Milton, NY (orchard 112: 54.7 ± 4.5) representing the minimum and maximum mean %RG, respectively, in this classification. Of the 34 sensitive orchard populations, 24 were commercial orchards where myclobutanil was applied for an average of 9 years, with approximately one to two applications per season. Three populations from research orchards that had received applications of DMI fungicides throughout the history of their planting and seven of the eight total baseline orchard populations were found to be sensitive to myclobutanil.

For the purpose of examining broader implications of myclobutanil resistance, orchard populations were sorted by their myclobutanil sensitivity classification according to the state and region from which they were collected (Table 2). Overall, 57, 69, and 15 populations of *V. inaequalis* were examined from the New England region (Connecticut, Maryland, Maine, New Hampshire, Rhode Island, and Vermont), the mid-Atlantic region (North Carolina, New York, Pennsylvania, Virginia, and West Virginia), and the Midwest region (Indiana, Michigan, Ohio, and Wisconsin), respectively. Although the majority of populations from each region were resistant to myclobutanil, the mid-Atlantic had the highest percentage of resistant populations (71%) followed by New England (56%) and the Midwest (53%). The Midwest had the greatest percentage of populations that were sensitive to myclobutanil (33%). Of the states (Maryland, Maine, New York, and Rhode Island) in which more than 10

Table 2. (continued from preceding column)

Classification, region, state ^a	Orchard populations			
	Myclobutanil		Difenoconazole	
	<i>n</i>	For state (%)	<i>n</i>	For state (%)
Mid-Atlantic				
Maryland	0	0	0	0
North Carolina	0	0	...	...
New York	5	9	1	7
Pennsylvania	0	0	...	...
Virginia	0	0	0	0
West Virginia	0	0	0	0
Midwest				
Indiana	0	0	...	...
Michigan	2	29	0	0
Ohio	0	0	...	...
Wisconsin	0	0	...	...
Total	18	13	1	3
Sensitive				
New England				
Connecticut	0	0	...	...
Massachusetts	1	6	1	100
Maine	9	45	13	100
New Hampshire	1	25	...	...
Rhode Island	4	29	...	...
Vermont	0	0	4	100
Mid-Atlantic				
Maryland	1	100	1	100
North Carolina	0	0	...	...
New York	12	21	13	93
Pennsylvania	0	0	...	...
Virginia	0	0	1	100
West Virginia	2	25	1	100
Midwest				
Indiana	1	100	...	...
Michigan	3	43	2	100
Ohio	1	16	...	...
Wisconsin	0	0	...	...
Total	34	24	36	97

populations of *V. inaequalis* were investigated, only Maine had more populations that were sensitive to myclobutanil (45%) than were resistant to myclobutanil (35%).

Baseline sensitivity and sensitivity of populations of *V. inaequalis* to difenoconazole. The EC_{50} values for baseline isolates of *V. inaequalis* to difenoconazole were 0.0002 to 0.009 $\mu\text{g ml}^{-1}$ (mean EC_{50} = 0.002 $\mu\text{g ml}^{-1}$) (Fig. 1). The majority (39 of 44) of isolates screened for baseline sensitivity had EC_{50} values below 0.004 $\mu\text{g ml}^{-1}$. Although a few isolates demonstrated a slight reduction in sensitivity to difenoconazole, no isolates tested had EC_{50} values that were either less than or greater than 10 $\times$ the mean (0.002 $\mu\text{g ml}^{-1}$).

From 2010 to 2013, 37 orchard populations of *V. inaequalis* throughout the northeastern United States were evaluated for sensitivity to difenoconazole (Table 3). Of these populations, 3 were from baseline orchards, 6 were from research orchards, and 28 were from commercial orchards. In total, 1,012 single-conidial lesion isolates of *V. inaequalis* were tested for sensitivity to difenoconazole, with 20 of the orchard populations being represented by 25 or more isolates. The remaining 17 orchard populations were represented by a minimum of 15 isolates.

None of the orchard populations was found to have practical resistance to difenoconazole, and only one population (orchard 148) was found to have reduced sensitivity to difenoconazole. The population with reduced sensitivity to difenoconazole was collected from a commercial orchard in which DMI fungicides had been used extensively and had a history of practical resistance to myclobutanil. This reduced-sensitive population had a mean %RG on difenoconazole-amended medium of $42.0 \pm 6.1\%$. Thirty-six remaining orchard populations were classified as being sensitive to difenoconazole. The mean %RG of sensitive orchard populations ranged from 3.2 to 41.1%, with a commercial orchard population in Sweden, ME (orchard 86: $3.2 \pm 1.2\%$) and a commercial orchard population in Geneva, NY (orchard 164: $41.1 \pm 2.9\%$) having the lowest and highest RG, respectively. Within all 29 commercial orchard populations, myclobutanil (Nova 40W or Rally 40WSP) was applied for an average of 13 years with two to four applications per season. Because difenoconazole in the form of Inspire Super (difenoconazole + cyprodinil; Syngenta) was first registered for use in New York in 2009, the product had only been available for use a maximum of four seasons, and most growers had only made one to two applications per season during those four seasons.

With the exception of a baseline orchard (orchard 28), all orchard populations were more sensitive to difenoconazole compared with myclobutanil and most individual isolates were more sensitive to difenoconazole than myclobutanil (data not shown). Of the 37 orchard populations surveyed for sensitivity to both fungicides, four

distinct phenotypic combinations were observed. In all, 14 orchard populations were found to be resistant to myclobutanil but sensitive to difenoconazole, 10 orchard populations had reduced sensitivity to myclobutanil but were sensitive to difenoconazole, 1 orchard population was resistant to myclobutanil and had reduced sensitivity to difenoconazole, and 12 orchard populations were sensitive to both fungicides (Tables 1 and 3). In a representative baseline population (orchard 28), some cross-sensitivity was observed between difenoconazole and myclobutanil ($R = 0.61$) (Fig. 2A). However, there was little to no cross-sensitivity between the two fungicides in a representative population with myclobutanil resistance (orchard 37) ($R = 0.07$) (Fig. 2B).

Impact of difenoconazole or myclobutanil fungicide applications on DMI fungicide sensitivity. In the Empire orchard, Inspire (difenoconazole) was applied a total of 16 times from 2010 to 2013. A decrease in sensitivity ("increase in resistance") to both myclobutanil and difenoconazole was observed in the population of *V. inaequalis* that was exposed to Inspire during this 4-year period (Fig. 3). Losses in sensitivity to myclobutanil increased steadily at a rate of 25% RG per year (Fig. 3). In 2010, the mean %RG of this population on medium amended with myclobutanil was $23.8 \pm 6.8\%$. By 2013, the population had developed practical resistance to myclobutanil, with a mean %RG of $96.4 \pm 2.2\%$. Although a loss of sensitivity to difenoconazole was also observed, percent changes in sensitivity during the 4-year period were not nearly as high as those for myclobutanil. In 2010, the isolate mean %RG on difenoconazole-amended medium was $19.0 \pm 8.9\%$ and, by 2013, the population was still sensitive to the fungicide, with an isolate mean %RG of $39.1 \pm 4.5\%$ (Fig. 3).

In vitro sensitivity to myclobutanil and difenoconazole was also determined for an orchard population with previously confirmed practical resistance to myclobutanil (Turechek et al. 2005). In the McIntosh and Cortland orchard from 2005 to 2010, myclobutanil (Rally 40WSP or Nova 40W) was applied 24 times. Interestingly, no significant difference in myclobutanil sensitivity was observed between the population evaluated in 2005 ($82.6 \pm 8.1\%$) and that evaluated in 2010 ($88.5 \pm 5.0\%$) (Fig. 4). In addition, when myclobutanil applications were terminated in the orchard (2011 to 2013), no significant difference in sensitivity to myclobutanil was observed compared with the years in which myclobutanil was applied in the orchard (2005 to 2010). Surprisingly, sensitivity to myclobutanil actually decreased from 2011 to 2013, with isolate mean %RG on myclobutanil-amended medium during those years increasing from 84.2 ± 5.3 to $93.2 \pm 2.2\%$. In vitro sensitivity to difenoconazole was lower than that of myclobutanil, ranging from 34.7 ± 4.1 to $44.3 \pm 2.2\%$ between 2009 and 2013, with a similar lack of fluctuation between years (Fig. 4).

Discussion

In all, 141 individual orchard populations of *V. inaequalis* from 16 states representing three major geographic regions were tested for sensitivity to the DMI fungicide myclobutanil over a period of 10 years. The majority of orchard populations were found to have practical resistance to the fungicide. Many of these orchards were commercial operations with an average of 13 applications of myclobutanil throughout the history of the orchard. Although myclobutanil may no longer be a reliable apple scab management option in orchards east of the Mississippi River, the use of newly released DMI fungicides such as difenoconazole may still be an effective alternative in management of apple scab. Indeed, nearly all populations of *V. inaequalis* tested for sensitivity to difenoconazole were found to be sensitive despite their phenotypic responses on myclobutanil-amended medium.

Resistance to myclobutanil and other DMI fungicides began appearing in commercial *V. inaequalis* populations as early as 1991 (Köller et al. 1997) and is now prevalent in major apple production regions, including those in Michigan, Virginia, Pennsylvania, Indiana, and Nova Scotia (Chapman et al. 2011; Köller et al. 1997; Marine et al. 2007; Pfeufer and Ngugi 2012). Although resistance to myclobutanil was previously detected in individual isolates of

Fig. 1. Distribution of difenoconazole EC_{50} values for 44 baseline isolates of *Venturia inaequalis*. Baseline isolates were collected from apple orchards in New York that have never been exposed to single-site fungicides.

V. inaequalis from New York (Köller et al. 1997), this is the first report of widespread myclobutanil resistance in the state and the New England region. Overall, 89 of 141 orchard populations across 16 states were confirmed to have practical resistance to myclobutanil. Because the distribution of myclobutanil sensitivity of isolates within each population was directly compared with a standard orchard population in which myclobutanil failed to adequately control apple scab, it is possible that myclobutanil could be ineffective in these 89 orchards, dependent on the susceptibility of the cultivar and weather during subsequent growing seasons.

Although the majority of orchard populations screened for myclobutanil sensitivity in this study were collected from orchards in which myclobutanil failed to provide adequate levels of apple scab control in the field, there were a few instances in which a field failure was reported but the population tested sensitive or reduced sensitive to myclobutanil in vitro. This was especially the case in 2009, in which there was low apple scab infection pressure through bloom throughout the northeastern United States, followed by favorable secondary apple scab conditions throughout the summer months. Dry conditions through bloom resulted in longer than average application

intervals and, as a result, minor primary apple scab infection events may have been missed. The weather across the region following petal fall and continuing throughout the summer months was wet and accompanied by below-average temperatures (Rosenberger et al. 2010). Usually during the summer, secondary apple scab infection slows because of a reduction of conidial production when temperatures surpass 26 to 29°C (MacHardy 1996). Because primary infections may have become established during the prebloom period, and because of the lack of consecutive days with warm temperatures to retard conidial production, the appearance of apple scab symptoms may have been mistaken for a fungicide control failure. Interestingly, several orchard populations evaluated for sensitivity in 2009 arrived for screening following petal fall. In other years of the survey, poor coverage or sprayer calibration may have resulted in fungicide control failures in the field rather than fungicide resistance.

Surprisingly, one baseline orchard without any historical applications of DMI fungicides was classified as reduced sensitive in this study. Although there are considerable numbers of wild apple trees in the region, the baseline orchard was fairly geographically isolated from commercial operations. Because the spatial spread of isolates of

Table 3. Sensitivity classification, percent relative growth, isolation year, and collection location from which *Venturia inaequalis* populations were surveyed for difenoconazole resistance

Orchard ^a	Classification ^b	n	%RG ^c	Pr > Ksa resistant ^d	Pr > Ksa sensitive ^d	Year	State
148	Red-sens.	21	42.0 ± 6.1	0.0750	0.8135	2011	New York
13	Sensitive	25	11.3 ± 1.5	<0.0001	<0.0001	2011	New York
19	Sensitive	26	24.8 ± 3.0	<0.0001	0.0863	2013	Vermont
26	Sensitive	35	35.0 ± 3.5	<0.0001	0.5187	2010	New York
28	Sensitive	17	16.0 ± 2.7	<0.0001	0.0006	2011	New York
37	Sensitive	35	39.1 ± 4.5	0.0011	0.2714	2013	New York
43	Sensitive	31	13.1 ± 2.1	<0.0001	0.0005	2012	Maine
61	Sensitive	36	21.9 ± 3.5	<0.0001	0.1131	2011	New York
86	Sensitive	16	3.2 ± 1.2	<0.0001	<0.0001	2013	Maine
88	Sensitive	35	22.3 ± 3.3	<0.0001	0.0023	2013	New York
101	Sensitive	22	15.1 ± 3.1	<0.0001	0.0071	2012	New York
104	Sensitive	23	5.7 ± 0.6	<0.0001	<0.0001	2011	Maine
110	Sensitive	34	29.7 ± 3.1	<0.0001	0.3625	2012	Vermont
119	Sensitive	19	11.1 ± 4.6	<0.0001	0.0003	2010	West Virginia
141	Sensitive	21	23.6 ± 6.1	0.0010	0.0911	2010	Maine
142	Sensitive	36	12.6 ± 1.8	<0.0001	<0.0001	2013	Michigan
147	Sensitive	23	37.6 ± 3.9	0.0013	0.8305	2012	New York
150	Sensitive	17	12.6 ± 2.7	<0.0001	0.0009	2011	Virginia
151	Sensitive	23	12.6 ± 2.4	<0.0001	0.0001	2011	Maine
152	Sensitive	25	24.8 ± 4.3	<0.0001	0.1736	2011	Maine
153	Sensitive	19	16.3 ± 3.5	<0.0001	0.0016	2011	Maine
157	Sensitive	32	27.3 ± 3.2	<0.0001	0.1464	2012	Vermont
158	Sensitive	24	10.1 ± 1.5	<0.0001	<0.0001	2011	Maine
161	Sensitive	15	20.1 ± 7.4	0.0002	0.0675	2010	New York
162	Sensitive	48	24.5 ± 2.8	<0.0001	0.0510	2012	Michigan
163	Sensitive	32	28.4 ± 3.6	<0.0001	0.3728	2012	Maine
164	Sensitive	37	41.1 ± 2.9	<0.0001	0.1042	2012	New York
165	Sensitive	19	15.8 ± 2.9	<0.0001	0.0032	2012	Massachusetts
166	Sensitive	36	4.26 ± 1.9	<0.0001	<0.0001	2012	Maine
167	Sensitive	35	10.0 ± 1.4	<0.0001	<0.0001	2012	Maine
168	Sensitive	23	4.5 ± 1.1	<0.0001	<0.0001	2012	Maine
169	Sensitive	25	13.7 ± 2.7	<0.0001	0.0002	2012	Maryland
170	Sensitive	33	29.6 ± 3.5	<0.0001	0.2034	2012	New York
171	Sensitive	35	37.8 ± 3.0	0.0002	0.5294	2012	New York
173	Sensitive	16	8.6 ± 3.3	<0.0001	0.0002	2013	Vermont
174	Sensitive	37	13.5 ± 2.9	<0.0001	<0.0001	2013	Maine
175	Sensitive	26	28.3 ± 5.1	0.0003	0.2565	2013	New York

^a Designation number represents the identical *V. inaequalis* population screened for resistance to myclobutanil.

^b Orchard classification was determined by comparing the distribution of difenoconazole sensitivity responses (isolate percent mean relative growth [%RG] values) for a given *V. inaequalis* orchard population with standard orchard populations with and without practical resistance to myclobutanil using the Kolmogorov-Smirnov two-sample test in SAS (version 9.3; SAS Institute, Cary, NC). Red-sens = reduced sensitive.

^c Orchard population mean percent growth of *V. inaequalis* isolates on medium amended with analytical-grade difenoconazole at 0.1 µg ml⁻¹ relative to that on nonfungicide medium (%RG). Values are means and standard errors of *n* isolates for each population, with five single-conidium colonies for each isolate.

^d Probability that the distribution of sensitivity responses for the orchard population in question is not significantly different from the standard orchard population with (Pr > Ksa resistant) or without (Pr > Ksa sensitive) practical resistance to myclobutanil.

V. inaequalis with insensitivity to DMI fungicides has been found previously to occur over a limited distance (Gao et al. 2009), the arrival of reduced-sensitive isolates of *V. inaequalis* most likely took a number of years. Given the number of wild hosts and the age of the baseline orchard (greater than 15 years), it is plausible that isolates with reduced sensitivity originated from a commercial orchard managed with DMI fungicides several kilometers away.

Maine was the only state surveyed in which the majority of its *V. inaequalis* populations were sensitive to myclobutanil. Although fungicide use practices were no different than in orchards in New York and other New England states, other factors such as climate may have contributed to preservation of myclobutanil efficacy in Maine. Although little is known about the effect of extended low temperatures on pseudothecia survival and viable ascospore emergence, cessation of pseudothecia and ascospore development has been observed at or below temperatures of 0°C. (Gadoury and MacHardy 1982). Indeed, extended subfreezing temperatures in Maine could result in reduced primary inoculum and, consequently, a lower absolute number of myclobutanil-resistant ascospores could have been released in the following spring. Marine et al. (2007) observed some reduced sensitivity to DMI fungicides when isolates of *V. inaequalis* were collected in July but not in cooler months of the spring. Another factor that may contribute to the greater number of sensitive populations in Maine is the relatively small orchard size

and low cultivar diversity within orchards (personal communication). Indeed, Pfeufer and Ngugi (2012) found that orchards smaller than 10 ha and those planted with fewer than 10 cultivars were significantly more sensitive to DMI fungicides.

In this study, the mean difenoconazole EC₅₀ value for 44 New York baseline isolates of *V. inaequalis* was determined to be 0.002 µg/ml. This concentration was considerably lower than previously reported EC₅₀ values for *V. inaequalis* baseline populations from Germany and Chile, which were 0.09 and 0.017 µg/ml, respectively (Henríquez et al. 2011; Kunz et al. 1997). Similarly, the wild-type *V. inaequalis* isolates used in other DMI baseline studies in North America also had a relatively higher level of variability in DMI sensitivity (Henríquez et al. 2011; Smith et al. 1991) than the baseline population selected for this study. In the current study, minimum and maximum EC₅₀ values of *V. inaequalis* isolates were separated by a factor of 45, with a corresponding resistance factor of 4.5 (data not shown). These factors are much lower than those reported for other DMI baseline studies on *V. inaequalis* and other fungal pathogens (Henríquez et al. 2011; Kunz et al. 1997; Smith et al. 1991; Spolti et al. 2014). The low frequency of isolates with reduced sensitivity to difenoconazole in our baseline population could have implications for the rate of development of resistance to difenoconazole. Within a given population, isolates with an EC₅₀ value above the mean for the population should have a greater likelihood for survival when difenoconazole is applied, compared with isolates more sensitive to difenoconazole. In the presence of a DMI fungicide, selection for such isolates with reduced sensitivity could ultimately lead to practical resistance over time. Thus, the paucity of isolates with reduced sensitivity to difenoconazole in our New York baseline population may favor a delayed onset of resistance to difenoconazole.

Of the 36 orchard populations that were confirmed to be sensitive to difenoconazole, 24 were either resistant or had reduced sensitivity to myclobutanil. Similarly, in our research orchard with stable practical resistance to myclobutanil, we found that isolates of *V. inaequalis* from that orchard population were sensitive to difenoconazole in each of 5 years tested. These observations were in agreement with previous studies that found that difenoconazole demonstrated greater intrinsic activity compared with other DMI fungicides (Henríquez et al. 2011; Kunz et al. 1997; Pfeufer and Ngugi 2012; Thomas et al. 2012). Although varying degrees of intrinsic activity among the DMI fungicides have been widely documented for a number of plant-pathogenic fungi (Köller et al. 1991; Wong and Midland 2007; Ypema et al. 1997), cross-sensitivity within this class generally

Fig. 2. Mean percent relative growth values of *Venturia inaequalis* isolates on difenoconazole at 0.1 µg/ml plotted against mean percent relative growth of those isolates on myclobutanil at 0.1 µg/ml. Isolates were collected from **A**, a baseline orchard or **B**, a research orchard resistant to myclobutanil. A linear distribution function [$y = (y_0 + ax)$] was fitted to the isolate mean percent relative growth values for myclobutanil and difenoconazole. For the baseline orchard, the R value was 0.610 ($n = 17$, $P = 0.0123$). For the myclobutanil-resistant orchard, the R value was 0.073 ($n = 35$, $P = 0.667$).

Fig. 3. Mean percent relative growth and standard errors of *Venturia inaequalis* isolates on myclobutanil (closed circles) or difenoconazole (open circles) at 0.1 µg/ml collected annually from a research orchard from 2010 to 2013. During each year, a minimum of 20 isolates of *V. inaequalis* were evaluated for sensitivity to each demethylation inhibitor (DMI) fungicide. Points where error bars did not overlap had significant differences in sensitivity between the two fungicides.

has historically been observed in baseline populations (Hsiang et al. 1997; Karaoglani and Thanassouloupoulos et al. 2003; Köller et al. 1991). In the current study, a positive correlation in the responses of *V. inaequalis* isolates to myclobutanil and difenoconazole was observed when the isolates were collected from a baseline orchard. However, this degree of cross-sensitivity was not evident in a population that previously had extensive exposure and was resistant to myclobutanil. A similar inconsistency in DMI cross-sensitivity had been observed in *Pyrenophora teres* for populations collected from different locations (Peever and Milgroom 1993). A lack of cross-sensitivity between two populations could be explained by differences in orchard microclimate, inherent differences in population composition (Xu et al. 2010), or type of management programs used. The absence of DMI cross-sensitivity for isolates in a myclobutanil-resistant population of *V. inaequalis* could also result from different genetic components being involved in resistance development for the two fungicides. Indeed, several mechanisms, including overexpression of the target *CYP51A1* gene (Schnabel and Jones 2001), point mutations within the *CYP51A1* gene (Délye et al. 1997), and overexpression of energy-dependent drug efflux pumps (Nakaune et al. 1998), have all been previously implicated in DMI fungicide resistance. In-depth examination into the molecular biology of *V. inaequalis* isolates collected from the same population with varying levels of sensitivity to myclobutanil and difenoconazole would be needed to test for the involvement of different genetic mechanisms of resistance.

Applications of myclobutanil or difenoconazole were made to populations of *V. inaequalis* displaying practical resistance or sensitivity to myclobutanil to examine changes in the distribution of isolate sensitivities to DMI fungicides. Twenty-four applications of myclobutanil applied to a *V. inaequalis* population over six seasons did not further shift the distribution of isolates toward greater levels of myclobutanil resistance. Moreover, the population did not shift toward myclobutanil sensitivity following the cessation of myclobutanil applications. In other fungal pathogens of fruit, including *Monilinia fructicola*, a fitness penalty has been associated with isolates that have reduced sensitivity to DMI fungicides (Cox et al. 2007). Although fitness penalties for DMI resistance have not been documented in *V. inaequalis*, we still suspect that periods without myclobutanil use (>20 years) could shift a population toward sensitivity. Indeed, populations of *V. inaequalis* formally resistant to dodine were recently found to be sensitive to the chemistry following

the curtailment of dodine use within an orchard for more than 20 years (Cox et al. 2010).

In our research orchard of 'Empire' apple with a myclobutanil-sensitive population of *V. inaequalis*, 16 applications of difenoconazole over a 4-year period resulted in increased population shifts toward myclobutanil and difenoconazole resistance. Interestingly, the level of resistance to myclobutanil in terms of the population mean increased at a steady rate of 25% RG per year compared with 6.7% RG for difenoconazole. Despite the discussion above about a lack of complete cross-sensitivity between myclobutanil and difenoconazole, we suspect that the field rate of difenoconazole may have quickly selected for isolates that were highly resistant to myclobutanil. Indeed, as a population becomes resistant to DMI fungicides, higher rates of a fungicide or applications of a more intrinsically active fungicide at an effectively similar rate may select for the most resistant members of a pathogen population (Lalancette et al. 1987). Although the population in our research orchard of 'Empire' apple was sensitive to myclobutanil in 2010, 20% of isolates tested in the orchard had RG values greater than 60% (data not shown). Because individual isolates of *V. inaequalis* with resistance to myclobutanil were already present in the orchard, even a single season of applications from the more intrinsically active difenoconazole may have quickly selected for highly resistant members.

Overall, results of this study have shown that practical resistance to myclobutanil is present in populations of *V. inaequalis* throughout the major apple-production regions in the northeastern United States. Unlike other fungicide classes (i.e., benzimidazoles and QoIs) where, qualitative resistance resulted in a relatively rapid emergence of resistance (<10 years of use) in the region, DMI fungicides like myclobutanil had been an essential component of apple scab management programs in the northeastern United States for more than 15 years. Although myclobutanil may no longer provide acceptable levels of apple scab control in most of the commercial orchards evaluated in this study, no orchard populations of *V. inaequalis* have practical resistance to difenoconazole. Still, caution should be exercised when applying difenoconazole in populations with resistance to myclobutanil, because such populations tend to have isolates with reduced sensitivity to difenoconazole.

Acknowledgments

This work was supported, in part, by funds appropriated to New York State Agricultural Experiment Station, Cornell University. We thank W. Köller and D. Parker for providing isolates, for training the authors on many of the techniques utilized in the microbiological assays of the study, and for providing useful advice on the analysis and interpretation of data; our collaborators who assisted in the collection of field samples and fungicide use histories for evaluated orchards, including J. Alicandro, P. Babcock, B. Baker, B. Bascombe, M. Belco, M. A. Bell, M. Biltonen, M. Borsick Herman, J. Bover, D. Breth, E. Brown, R. Bubier, J. Carroll, A. Clark, S. Clark, L. DeVoe, J. Dinardo, R. Drown, M. Ellis, M. Fargione, H. Faubert, H. Feller, M. Forrence, M. Forrence, M. Funk, Z. Goodband, D. Green III, A. Grobe, D. Hartley, L. Herring, A. Hill, C. Hodges, J. Iverson, C. Jaswell, E. Jensen, K. Karter, G. Koehler, E. Leadbeater, J. Lyman, A. Lynd, J. Matthews, J. Mitsiti, G. Morin, D. O'Connor, B. Olson, C. Petzoldt, D. Polseno, D. Rainer, D. Rosenberger, F. Santurri, R. Seem, M. Shannon, D. Simmons, B. Smith, R. Spitko, J. Steere, A. Sullivan, A. Teach, P. TenEyck, G. Thornton, D. Tower, F. Trail, B. Truncali, A. Tuttle, R. Wander, A. Wodehouse, S. Wood, M. Wright, K. Yoder, M. Zifchock, and S. Zonneville; and K. Abbot, M. Ellis, J. Freier, N. Gottschall, C. Lehman, C. K. Poon, M. Rosato, and K. Villani for their assistance in the collection and preparation of and evaluation of isolates.

Literature Cited

- Beckerman, J. L., Sundin, G. W., and Rosenberger, D. A. 2015. Do some IPM concepts contribute to the development of fungicide resistance? Lessons learned from the apple scab pathosystem in the United States. *Pest Manage. Sci.* 71:331-342.
- Braun, P. G., and McRae, K. B. 1992. Composition of a population of *Venturia inaequalis* resistant to myclobutanil. *Can. J. Plant Pathol.* 14:215-220.
- Chapman, K. S., Sundin, G. W., and Beckerman, J. L. 2011. Identification of resistance to multiple fungicides in field populations of *Venturia inaequalis*. *Plant Dis.* 95:921-926.
- Cox, K. D., Bryson, P. K., and Schnabel, G. 2007. Instability of propiconazole resistance and fitness in *Monilinia fructicola*. *Phytopathology* 97:448-453.
- Cox, K. D., Villani, S. M., Ellis, M. A., Poon, C. K., and Craig-Kuhn, M. C. 2009. Evaluation of fungicides and application timing for control of powdery mildew and apple scab, 2008. *Plant Dis. Manage. Rep.* 3:PF038. Online publication. doi:10.1094/PDMR03

Fig. 4. Mean percent relative growth of *Venturia inaequalis* isolates collected from a research orchard from 2005 to 2013 to the demethylation inhibitor (DMI) fungicides myclobutanil (black bars) and difenoconazole (gray bars). Difenoconazole sensitivity was not evaluated from 2005 through 2008. From 2005 to 2010, myclobutanil was applied four times per season, and applications were discontinued in 2011. Number of isolates (*n*) tested for sensitivity to myclobutanil or sensitivity to difenoconazole in each year were 2005: *n* = 30, 2006: *n* = 30, 2007: *n* = 30, 2008: *n* = 50, 2009: *n* = 32, 2010: *n* = 29, 2011: *n* = 29, 2012: *n* = 35, and 2013: *n* = 35.

- Cox, K. D., Villani, S. M., and Jacon, G. 2010. Status of dodine resistance and possibilities for renewed use against *Venturia inaequalis* populations in the northeastern U.S. (Abstr.) Phytopathology 100:S27.
- Cox, K. D., Villani, S. M., and Raes, J. J. 2013. Evaluation of fungicides for the control of apple scab and powdery mildew in an 'Empire'-'Jonagold' orchard with a DMI and QoI-resistant apple scab population, 2012. Plant Dis. Manage. Rep. 7:PF003. Online publication. <http://www.plantmanagementnetwork.org/pub/trial/pdmr/volume7/abstracts/pf003.asp>
- Délye, C., Laigre, F., and Corio-Costet, M.-F. 1997. A mutation in the 14 α -demethylase gene of *Uncinula necator* that correlates with resistance to a sterol biosynthesis inhibitor. Appl. Environ. Microbiol. 63:2966-2970.
- Frederick, Z. A., Villani, S. M., Cooley, D. R., Biggs, A. R., Raes, J. J., and Cox, K. D. 2014. Prevalence and stability of qualitative QoI resistance in populations of *Venturia inaequalis* in the northeastern United States. Plant Dis. 98:1122-1130.
- Gadoury, D. M., and MacHardy, W. E. 1982. A model to estimate the maturity of ascospores of *Venturia inaequalis*. Phytopathology 72:901-904.
- Gao, L., Berrie, A., Yang, J., and Xu, X. 2009. Within- and between-orchard variability in the sensitivity of *Venturia inaequalis* to myclobutanil, a DMI fungicide, in the UK. Pest Manage. Sci. 65:1241-1249.
- Henríquez, J. L., Sarmiento V. O., and Alarcón, P. 2011. Sensitivity of *Venturia inaequalis* Chilean isolates to difenoconazole, fenarimol, mancozeb, and pyrimethanil. Chil. J. Agric. Res. 71:39-44.
- Hildebrand, P. D., Lockhart, C. L., Newbery, R. J., and Ross, R. G. 1988. Resistance of *Venturia inaequalis* to bitertanol and other demethylation-inhibiting fungicides. Can. J. Plant Pathol. 10:311-316.
- Hsiang, T., Yang, L., and Barton, W. 1997. Baseline sensitivity and cross-resistance to demethylation-inhibiting fungicides in Ontario isolates of *Sclerotinia homoeocarpa*. Eur. J. Plant Pathol. 103:409-416.
- Jobin, T., and Carisse, O. 2007. Incidence of myclobutanil- and kresoxim-methyl-insensitive isolates of *Venturia inaequalis* in Quebec orchards. Plant Dis. 91:1351-1358.
- Jones, A. L. 1981. Fungicide resistance: Past experience with benomyl and dodine and future concerns with sterol inhibitors. Plant Dis. 65:990-992.
- Karaoglanidis, G. S., and Thanassouloupoulos, C. C. 2003. Cross-resistance patterns among sterol biosynthesis inhibiting fungicides (SBIs) in *Cercospora beticola*. Eur. J. Plant Pathol. 109:929-934.
- Köller, W. 1991. Fungicide resistance in plant pathogens. Pages 679-720 in: CRC Handbook of Pest Management in Agriculture, Vol. 2, 2nd ed. D. Pimentel, ed. CRC Press, Boca Raton, FL.
- Köller, W., Parker, D. M., and Reynolds, K. L. 1991. Baseline sensitivities of *Venturia inaequalis* to sterol demethylation inhibitors. Plant Dis. 75:726-728.
- Köller, W., Parker, D. M., Turechek, W. W., Avila-Adame, C., and Cronshaw, K. 2004. A two-phase resistance response of *Venturia inaequalis* populations to the QoI fungicides kresoxim-methyl and trifloxystrobin. Plant Dis. 88:537-544.
- Köller, W., and Wilcox, W. F. 1999. Evaluation of tactics for managing resistance of *Venturia inaequalis* to sterol demethylation inhibitors. Plant Dis. 83:857-863.
- Köller, W., and Wilcox, W. F. 2001. Evidence for the predisposition of fungicide-resistant phenotypes of *Venturia inaequalis* to a preferential selection for resistance to other fungicides. Phytopathology 91:776-781.
- Köller, W., Wilcox, W. F., Barnard, J., Jones, A. L., and Braun, P. G. 1997. Detection and quantification of resistance of *Venturia inaequalis* populations to sterile demethylation inhibitors. Phytopathology 87:184-190.
- Kunz, S., Deising, H., and Mendgen, K. 1997. Acquisition of resistance to sterol demethylation inhibitors by populations of *Venturia inaequalis*. Phytopathology 87:1272-1278.
- Lalancette, N., Hickey, K. D., and Cole, H., Jr. 1987. Effects of mixtures of benomyl and mancozeb on the buildup of benomyl-resistant *Venturia inaequalis*. Phytopathology 77:86-91.
- Lesniak, K. E., Proffer, T. J., Beckerman, J. L., and Sundin, G. W. 2011. Occurrence of QoI resistance and detection of the G143A mutation in Michigan populations of *Venturia inaequalis*. Plant Dis. 95:927-934.
- MacHardy, W. E. 1996. Apple Scab—Biology, Epidemiology, and Management. American Phytopathological Society, St. Paul, MN.
- MacHardy, W. E., and Gadoury, D. M. 1989. A revision of Mills' s criteria for predicting apple scab infection periods. Phytopathology 79:304-310.
- MacHardy, W. E., Gadoury, D. M., and Gessler, C. 2001. Parasitic and biological fitness of *Venturia inaequalis*: Relationship to disease management strategies. Phytopathology 85:1036-1051.
- Marine, S. C., Schmale, D. G., and Yoder, K. S. 2007. Resistance to myclobutanil in populations of *Venturia inaequalis* in Winchester, Virginia. Online publication. Plant Health Prog. doi:10.1094/PHP-2007-1113-01-RS
- Nakaune, R., Adachi, K., Nawata, O., Tomiyama, M., Akutsu, K., and Hibi, T. 1998. A novel ATP-binding cassette transporter involved in multidrug resistance in the phytopathogenic fungus *Penicillium digitatum*. Appl. Environ. Microbiol. 64:3983-3988.
- O'Leary, A. L., Jones, A. L., and Ehret, G. R. 1987. Application rates and spray intervals for apple scab control with flusilazol and pyrifeno. Plant Dis. 71:623-626.
- Peever, T. L., and Milgroom, M. G. 1993. Genetic correlations in resistance to sterol biosynthesis-inhibiting fungicides in *Pyrenophora teres*. Phytopathology 83:1076-1082.
- Pfeuffer, E. E., and Ngugi, H. K. 2012. Orchard factors associated with resistance and cross resistance to sterol demethylation inhibitor fungicides in populations of *Venturia inaequalis* from Pennsylvania. Phytopathology 102:272-282.
- Rosenberger, D. A., Cox, K. D., Rugh, A., Villani, S. M., and Frederick, Z. 2010. Apple scab management options for high inoculum orchards. N. Y. Fruit Q. 20:9-13.
- Schnabel, G., and Jones, A. L. 2001. The 14 α -Demethylase (*CYP51A1*) gene is overexpressed in *Venturia inaequalis* strains resistant to myclobutanil. Phytopathology 91:102-110.
- Schwabe, W. F. S., Jones, A. L., and Jonker, J. P. 1984. Greenhouse evaluation of the curative and protective action of sterol-inhibiting fungicides against apple scab. Phytopathology 74:249-252.
- Smith, F. D., Parker, D. M., and Köller, W. 1991. Sensitivity distribution of *Venturia inaequalis* to the sterol demethylation inhibitor flusilazole: Baseline sensitivity and implications for resistance monitoring. Phytopathology 81:392-396.
- Spolti, P., Del Ponte, E. M., Dong, Y., Cummings, J. A., and Bergstrom, G. C. 2014. Triazole sensitivity in a contemporary population of *Fusarium graminearum* from New York wheat and competitiveness of a tebuconazole-resistant isolate. Plant Dis. 98:607-613.
- Stanis, V. F., and Jones, A. L. 1985. Reduced sensitivity to sterol-inhibiting fungicides in field isolates of *Venturia inaequalis*. Phytopathology 75:1098-1101.
- Stenzel, K. 2014. FRAC SBI Working Group. Online publication. <http://www.frac.info/working-group/sbi-fungicides>
- Szokolnik, M. 1981. Physical modes of action of sterol-inhibiting fungicides against apple diseases. Plant Dis. 65:981-985.
- Thomas, A., Langston, D. B., Jr., and Stevenson, K. L. 2012. Baseline sensitivity and cross-resistance to succinate-dehydrogenase-inhibiting and demethylation-inhibiting fungicides in *Didymella bryoniae*. Plant Dis. 96:979-984.
- Turechek, W. W., Heidenreich, M. M., and Heidenreich, G. 2005. Evaluation of captan fungicide programs for management of apple scab in a benzimidazole and DMI-resistant orchard, 2004. Fungic. Nematicide Tests 60:PF027. Online Publication. doi:10.1094/FN60
- Villani, S. M., and Cox, K. D. 2011. Characterizing fenbuconazole and propiconazole sensitivity and prevalence of 'Mona' in isolates of *Monilinia fructicola* from New York. Plant Dis. 95:828-834.
- Villani, S. M., and Cox, K. D. 2014. Heteroplasmy of the *cytochrome b* gene in *Venturia inaequalis* and its involvement in quantitative and practical resistance to trifloxystrobin. Phytopathology 104:945-953.
- Wong, F. P., and Midland, S. L. 2007. Sensitivity distributions of California populations of *Colletotrichum cereale* to the DMI fungicides propiconazole, myclobutanil, tebuconazole, and triadimefon. Plant Dis. 91:1547-1555.
- Xu, X.-M., Gao, L.-Q., and Yang, J.-R. 2010. Are insensitivities of *Venturia inaequalis* to myclobutanil and fenbuconazole correlated? Crop Prot. 29:183-189.
- Yoshimura, M. A., Luo, Y., Ma, Z., and Michailides, T. J. 2004. Sensitivity of *Monilinia fructicola* from stone fruit to thiophanate-methyl, iprodione, and tebuconazole. Plant Dis. 88:373-378.
- Ypema, H. L., Ypema, M., and Gubler, W. D. 1997. Sensitivity of *Uncinula necator* to benomyl, triadimefon, myclobutanil, and fenarimol in California. Plant Dis. 81:293-297.