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Managing Resistance of *Venturia inaequalis* to the Strobilurin Fungicides

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Abstract

Over the past thirty years, fungicide resistance has been cited as the cause of large-scale crop losses due to apple scab in a number of commercial orchards in the Northeast. The strobilurins represent the most recent class of fungicides for which the threat of resistance exists. In this article we examine the use of strobilurin fungicides at their highest and lowest labeled rates and as a mixture of the lowest labeled rate with a protectant fungicide in commercial spray programs for efficacy against apple scab as well as their utility in a resistance management program. Our results suggest that anti-resistance properties of high strobilurin rates are superior to low rates and low rate mixtures with a protectant fungicide.

Introduction

Apple growers consider the economy, efficacy, and (less often) the potential for the development of resistance when selecting fungicides for managing apple scab. Numerous apple growers have lost or are in danger of losing several classes of highly effective scab fungicides due to the development of resistance. These include dodine, the benzimidazoles (benlate and thiophanate-methyl), and, most recently, the class of demethylation-inhibiting (DMI) fungicides, including fenarimol (Rubigan E.C.), myclobutanil (Nova 40W), and triflumizole (Procure 50WS) (2). The DMI fungicides, although still effective in many orchards, are gradually losing their efficacy due to quantitative sensitivity shifts in populations of *V. inaequalis* (4,7). The strobilurin fungicides represent a more recent class of chemistry available to growers for management of apple scab, with trifloxystrobin (Flint 50WG) and kresoxim-methyl (Sovran 50WG) registered for current use in the U.S.

The strobilurins, also named Qo inhibitors or QoIs for short, were introduced in the mid-1990s. They exhibit efficacy against a broad-spectrum of fungal diseases, possess significant postinfective activity, and have a unique mode of action (1). Several pathogens have developed qualitative resistance to the strobilurins caused by a G143A mutation of the cytochrome *b* target site (1). This mechanism of resistance was first described for a laboratory mutant of *V. inaequalis* (6,10) and more recently for an apple orchard in Germany (8), but similar mutants have, thus far, not been identified in the United States. Instead, gradual quantitative shifts of population sensitivities to strobilurins are being discovered (8). It was speculated that such initially quantitative population responses might facilitate the generation of immune G143A mutants (8). Consequently, slowing the speed of initially quantitative shifts might be expected to slow the emergence of qualitative resistance based on the generation and subsequent selection of immune target site mutants.

For the class of DMI fungicides with their quantitative pattern of resistance development (7), high rates of application continued to provide disease control of the least sensitive isolates, thus slowing the speed of their selection (5). A mixture of the protectant mancozeb with a DMI at a low rate failed to slow the speed of selection. Instead, control of DMI-resistant isolates was only provided by the mixing partner mancozeb. It was thus recommended to combine both resistance management strategies: High label rates of DMIs in order to slow the speed of resistance development in mixture with a protectant to provide additional control of resistant isolates (5).

Fig. 1. **A** and **B**. Apple scab symptoms on (A) a cluster leaf and (B) a terminal leaf. **C**. Old or exhausted apple scab lesions on a terminal leaf. **D**. Young lesion on a developing fruit. **E** and **F**. Well-developed lesions (E) on a mature fruit with characteristic cracking shown in F.

The present study was designed to evaluate the utility of adding a protectant fungicide as a mixing partner with the strobilurin fungicides for: (i) improving control of apple scab; and (ii) managing fungicide resistance. The importance of recognizing the functional role of each mixing partner is emphasized.

Efficacy Trials

Trials were conducted in research orchards on the property of the New York State Agricultural Experiment Station in Geneva, NY. In 2002, trials were conducted in an orchard comprised of 27-year-old 'Cortland' trees planted on MM.106 rootstock. In 2003, trials were conducted in an orchard comprised of 28-year-old 'McIntosh' trees on MM.106 rootstock and in an 8-year-old orchard comprised of 'Empire' and 'Jonagold' trees planted on MM.111 rootstock with an M.9 interstem. DMI-resistant isolates of *Venturia inaequalis* are prevalent in both the McIntosh and Cortland orchard blocks (8). The status of DMI-resistance in the Empire orchard is unknown.

Single-tree plots, spaced 7.6 m apart within rows, were used in both the Cortland and McIntosh orchards. In the Empire/Jonagold orchard, trees were planted in alternation within rows such that individual plots consisted of two trees, one 'Empire' and one 'Jonagold'. In all orchards, treatments consisted of 4 replications arranged in a randomized complete block. Treatments began with one or two applications of mancozeb (Dithane RSNT) corresponding with the availability of mature ascospores and suitable climatic conditions. To comply with label restrictions, four strobilurin applications per season were integrated into a rotational scab management program, with the first application at tight cluster followed by applications at pink or early bloom, petal fall and ending with the first cover spray (Table 1). The strobilurins were applied: (i) singly at their highest labeled rates; (ii) singly at their lowest labeled rates; and (iii) as mixtures at their lowest labeled rates with mancozeb, also at the low label rate. All treatments were applied to drip using a handgun at a pressure of 2414 kPa. Additional cover sprays of Captan and thiophanate-methyl (Topsin-M) were applied with an airblast sprayer at a volume of water approximately one-third of that necessary for drip.

Table 1. Fungicide application timings for three efficacy trials conducted in experimental orchards at the NY State Agricultural Experiment Station. Asterisks denote application dates that included a strobilurin fungicide.

Apple growth stage	Variety and year of experiment		
	Cortland 2002	McIntosh 2003	Empire 2003
Green tip	--	April 16	April 16
Half-inch green	April 18	April 24	April 24
Tight cluster	April 24*	April 30*	April 30*
Pink	May 1	May 7*	May 7*
Bloom	May 8*, 15	May 15	May 15
Petal fall	May 21*	May 22*	May 22*
First cover	May 29*	May 29*	May 29*
Second cover	June 13	June 10	June 10
Third cover	June 26	June 23	June 23
Fourth cover	July 11	July 8	July 8
Fifth cover	July 25	July 16	July 16
Sixth Cover	August 7	July 23	July 25
Seventh Cover	--	August 8	August 8

Table 2. Fungicide performance in the management of apple scab in 2002 on 'Cortland' in an experimental orchard in Geneva, New York.

Fungicide	Rate ^w (g a.i./ha)	Terminal leaves		Fruit scab	
		Incidence ^x	Control (%) ^y	Incidence	Control (%)
Kresoxim methyl	220	16.5 cd	79.0	2.0 de	97.8
Kresoxim methyl	140	18.5 cd	76.4	3.7 de	96.0
Kresoxim methyl + mancozeb	140 2500	14.7 d	81.3 (87.2) ^z	0.5 e	99.5 (99.2)
Trifloxystrobin	105	16.0 cd	79.6	3.7 de	96.0
Trifloxystrobin	70	15.2 cd	80.6	5.0 de	94.6
Trifloxystrobin + mancozeb	70 2500	22.5 cd	71.3 (89.4)	6.2 cd	93.3 (99.0)
Mancozeb	2500	42.7 b	45.6	17.7 bc	80.9
Myclobutanil	126	26.7 c	66.0	21.8 b	76.5
UTC	--	78.5 a	--	92.9 a	--

^w Rate is based on 2800 liter/ha.

^x Mean percent disease values from four replicate plots per treatment from disease ratings on August 16, 2002. Means within a column not followed by a common letter are significantly different from each other according to the pairwise difference option (PDIF) in PROC MIXED ($P < 0.05$).

^y Percentages of control were calculated as:
 $100 - \text{incidence (treated)} / \text{incidence (untreated)}$.

^z Expected additive effects (Expected = $A + B - [AB/100]$).

Table 3. Fungicide performance in the management of apple scab in 2003 on 'McIntosh' in an experimental orchard in Geneva, New York.

Fungicide	Rate ^w (g a.i./ha)	Terminal leaves		Fruit scab	
		Incidence ^x	Control (%) ^y	Incidence	Control (%)
Kresoxim methyl	220	8.0 d	89.8	7.8 e	91.8
Kresoxim methyl	140	13.8 d	82.4	13.0 de	86.3
Kresoxim methyl + mancozeb	140 2500	15.5 d	80.3 (92.7) ^z	6.8 e	92.8 (93.7)
Trifloxystrobin	105	4.8 c	93.9	9.5 de	90.0
Trifloxystrobin	70	13.5 d	82.8	17.8 de	81.3
Trifloxystrobin + mancozeb	70 2500	14.8 d	81.1 (92.9)	12.0 de	87.4 (91.4)
Mancozeb	2500	32.5 bc	58.6	43.8 bc	53.9
Myclobutanil	126	36.5 b	53.5	57.0 b	40.0
UTC	--	78.5 a	--	95.0 a	--

^w Rate is based on 2800 liter/ha.

^x Mean percent disease values from four replicate plots per treatment from disease ratings on August 18, 2003. Means within a column not followed by a common letter are significantly different from each other according to the pairwise difference option (PDIFF) in PROC MIXED ($P < 0.05$).

^y Percentages of control were calculated as:
100 – incidence (treated) / incidence (untreated).

^z Expected additive effects (Expected = A+B - [AB/100]).

Table 4. Fungicide performance in the management of apple scab in 2003 on 'Empire' in an experimental orchard in Geneva, New York.

Fungicide	Rate ^w (g a.i./ha)	Terminal leaves		Fruit scab	
		Incidence ^x	Control (%) ^y	Incidence	Control (%)
Kresoxim methyl	220	1.5 c	97.7	0.5 b	99.4
Kresoxim methyl	140	1.3 c	98.0	3.0 b	96.1
Kresoxim methyl + mancozeb	140 2500	0.8 c	98.8 (99.8) ^z	1.5 b	98.1 (99.7)
Trifloxystrobin	105	1.3 c	98.0	1.0 b	98.7
Trifloxystrobin	70	0.8 c	98.8	4.5 b	94.2
Trifloxystrobin + mancozeb	70 2500	1.5 c	97.7 (99.9)	0.5 b	99.4 (99.5)
Mancozeb	2500	7.0 b	89.1	6.0 b	92.3
Myclobutanil	126	9.8 b	84.8	12.5 b	83.9
UTC	--	64.3 a	--	77.5 a	--

^w Rate is based on 2800 liter/ha.

^x Mean percent disease values from four replicate plots per treatment from disease ratings on August 20, 2003. Means within a column not followed by a common letter are significantly different from each other according to the pairwise difference option (PDIFF) in PROC MIXED ($P < 0.05$).

^y Percentages of control were calculated as:
100 – incidence (treated) / incidence (untreated).

^z Expected additive effects (Expected = A+B - [AB/100]).

Apple scab incidence (presence/absence) was evaluated by examining all leaves on the 10 oldest fully expanded leaves on each of 10 terminal branches, and on 50 or 100 fruit per tree on the dates indicated in Tables 2, 3, and 4. Data were transformed using the arcsin transformation. Transformed data were analyzed by ANOVA using SAS PROC MIXED and treatment means were separated using the pairwise difference option (PDIFF) in PROC MIXED ($P < 0.05$).

Results further document the efficacy of the strobilurin fungicides for managing apple scab under heavy disease pressure. Moreover, strobilurins remain highly effective in the management of DMI-resistant orchard populations of *V. inaequalis*, as exemplified by the Cortland and McIntosh blocks reported previously as DMI-resistant (Tables 2 and 3) (8). Although not tested directly, the *V. inaequalis* population at the Empire block appears to be DMI-resistant, as indicated by the failure of myclobutanil to improve scab control above the level achieved with the protectant mancozeb alone (Table 4). In general, disease management was more effective in the Empire orchard (Table 4) versus the Cortland and McIntosh orchards (Tables 2 and 3), because the trees were younger and smaller (being grafted on to a dwarfing rootstock), consequently, spray coverage was more thorough. Additionally, disease pressure was perhaps lower because the orchard had not been subjected to the same degree of intentional inoculum build-up as the McIntosh and Cortland blocks.

In all three trials, the performance of both strobilurins was numerically improved at high application rates (Tables 2, 3, and 4). The same improvement was achieved by mixing strobilurins at their low rates with mancozeb (Tables 2, 3, and 4). However, the level of control achieved was consistently lower than expected from the purely additive effects of the mixture components, as calculated by Additive Effects = $A + B - (AB/100)$, with A and B as the percentage of control achieved with each of the mixture components alone (9). A reason for the slightly antagonistic action of mancozeb in mixture with trifloxystrobin remains unclear.

Sensitivity Shifts

As reported recently, quantitative responses of the *V. inaequalis* population to strobilurin treatments were documented in 2002 for the Cortland block, although respective sensitivity shifts had no apparent effect on strobilurin performance (8). In 2003, respective sensitivity shifts were evaluated for the McIntosh block (adjacent to the Cortland block) employing the same test procedure described previously (8). In brief, individual scab lesions were excised, and conidia were dispensed in water and allowed to germinate and develop mycelia for seven days on potato dextrose agar without fungicide or amended with 0.02 µg a.i./ml of the strobilurin trifloxystrobin. Sensitivities were measured as relative growth (RG) of mycelia (growth amended/growth unamended $\times 100$).

The results were consistent with data reported for 2002 (8). In comparison to a composite baseline population collected from five orchards never treated with any fungicide, the relative frequency of isolates at the McIntosh block had shifted significantly ($P < 0.001$) towards decreased trifloxystrobin sensitivity (Fig. 1). As documented previously (8), however, this magnitude of sensitivity shifts had no apparent impact on satisfactory scab performances of strobilurins (Table 3).

Fig. 2. Frequency distribution of trifloxystrobin sensitivities of *Venturia inaequalis* isolates for a composite baseline population never treated with trifloxystrobin (solid bars; $n=195$; mean relative growth = 3.2) and for the population at an experimental McIntosh orchard (hatched bars; $n=62$; mean relative growth = 23.1). Mean relative growth (RG) = growth amended/growth unamended $\times 100$ at a discriminatory dose of 0.02 $\mu\text{g/ml}$ trifloxystrobin.

In order to assess the population response to treatments with the low and high rates of trifloxystrobin and the trifloxystrobin:mancozeb mixture, diseased leaves in the McIntosh block were collected from non-treated check-trees, and from trees treated with trifloxystrobin. In order to allow full population responses to season-long trifloxystrobin programs, diseased leaves were sampled two weeks after the last trifloxystrobin application. All three treatments with trifloxystrobin had highly significant effects on the mean RG values and the frequency distributions of isolate sensitivities (Table 5), indicating significant population responses to trifloxystrobin. Among the three treatment options, differences were not highly significant ($P > 0.05$). Isolates exhibiting RG values > 50 had been selected most prominently, with the mixture of trifloxystrobin and mancozeb mandating the lowest increase in frequencies (Table 5). Highly resistant isolates indicative of target site mutants were not identified in any of the populations tested, indicating that all population responses were quantitative in character.

Table 5. Responses of *Venturia inaequalis* populations to treatments with trifloxystrobin in 2003 ('McIntosh').

Fungicide	Rate ^w (g a.i./ha)	Population sensitivities			Isolate frequencies (%)	
		n	RG ^x	Pm^y	RG > 50	P^z
UTC	--	62	23.0		1.6	
Trifloxystrobin	70	40	38.2	<0.001	17.5	0.003
Trifloxystrobin	105	49	32.8	0.003	10.2	0.04
Trifloxystrobin + mancozeb	70 2500	51	32.0	0.002	7.8	0.1

^w Rate is based on 2800 liter/ha.

^x Mean relative growth (RG) of mycelia (growth amended/growth unamended $\times 100$) at a discriminatory dose of 0.02 $\mu\text{g/ml}$ trifloxystrobin.

^y Comparison of mean RG values (t test).

^z Comparison of isolate frequencies exhibiting RG values > 50 (log-linear analysis).

The quantitative population responses to trifloxystrobin were determined by calculating the levels of scab control achieved for infections caused by the two subpopulations of *V. inaequalis* exhibiting RG values ≤ 50 and > 50 . The proportion of disease caused by isolates belonging to the two subpopulations were calculated from the total disease incidence (Table 3) and the frequencies of isolates exhibiting RG > 50 (Table 5), according to a procedure employed previously (5).

The subpopulation of isolates with RG values > 50 were not controlled at the low rate of trifloxystrobin, while the level of control achieved for sensitive isolates with RG ≤ 50 was high for both fruit and leaf scab (Table 6). In contrast, trifloxystrobin at a high dose continued to provide measurable control of isolates with RG values > 50 (Table 6). The subpopulation exhibiting RG values > 50 was also less effectively controlled by the mixture of trifloxystrobin at the low rate with mancozeb and the control achieved with the mixture was considerably lower than the level expected from additive effects of the two fungicides in mixture (Table 6).

Table 6. Differential control of *Venturia inaequalis* isolates with different sensitivities to trifloxystrobin ('McIntosh,' 2003).

Fungicide	Rate ^w (g a.i./ha)	Terminal leaf scab incidence ^x	
		RG ^y ≤ 50	RG > 50
Untreated check	--	77.2	1.3
Trifloxystrobin	70	11.1 [86]	2.4 [0]
Trifloxystrobin	105	4.3 [94]	0.5 [62]
Trifloxystrobin + mancozeb	70 2500	13.7 [82] [94] ^z	1.1 [15] [59] ^z

^x Rate is based on 2800 liter/ha.

^y Disease incidences caused by isolates displaying respective RG values were calculated from values given in Table 3. Numbers in parentheses reflect the percentage of control.

^z Mean relative growth (RG) of mycelia (growth amended/growth unamended $\times 100$) at a discriminatory dose of 0.02 $\mu\text{g/ml}$ trifloxystrobin.

^z Expected additive effect (Expected = $A + B - [AB/100]$) calculated from the mancozeb performance data listed in Table 3.

Conclusion

The results presented in this study have impact on the management of *V. inaequalis* resistance to strobilurins. In general, the pathway of resistance can be a qualitative selection of immune subpopulations, as exemplified by resistance to the benzimidazoles, or a quantitative and gradual response of populations as is most thoroughly documented for the DMI fungicides (3). In the latter case of quantitative resistance, control of resistant isolates remains dependent on the fungicide dose, allowing a decrease in the speed of resistance development at high doses (5). For the strobilurins, the path of resistance development was shown to follow a dual path, with a quantitative population response of *V. inaequalis* populations followed by the selection of immune G143A target site mutants (8). Circumstantial evidence allowed speculation that (i) quantitative resistance as such can lead to unsatisfactory control of scab, and that (ii) slowing the speed of initially quantitative population responses might slow the subsequent phase of qualitative resistance development (8).

As for the DMIs with their quantitative development of resistance (5), our preliminary data indicate that a higher application rate of strobilurins will continue to provide control of isolates capable of escaping control at the lowest recommended rate, i.e., isolates with RG values > 50 (Table 6). High application rates can thus be expected to slow the speed of initially quantitative population responses toward strobilurin resistance. A mixture of trifloxystrobin at its lowest labeled rate with the protectant mancozeb also provided improved control of the

subpopulation escaping from trifloxystrobin alone. However, the level of control was below the expected level of control achieved with mancozeb alone (Table 6). This slight antagonistic effect might diminish the effectiveness of the mixture in slowing the build-up of strobilurin-resistant subpopulations. Our results suggest that anti-resistance properties of high strobilurin rates are superior to low rate mixtures with mancozeb.

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