Evaluation of Tactics for Managing Resistance of *Venturia inaequalis* to Sterol Demethylation Inhibitors

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ABSTRACT

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The impact on the selection and control of subpopulations of *V. inaequalis* resistant to the sterol demethylation inhibitor (DMI) fenarimol or to dodine were evaluated with respect to several tactics of apple scab control. Experiments were conducted in an experimental orchard with elevated levels of DMI and dodine resistance over a period of three consecutive seasons. The DMI-resistant subpopulation was poorly (14%) controlled at a fenarimol rate of 15 mg/liter (sprayed to run-off), whereas control was significantly improved (54%) at twice that rate. Mancozeb mixed with the low rate of fenarimol also improved the control of DMI-resistant isolates, but the improvement was due to the indiscriminate control of both the DMI-sensitive and -resistant populations provided by mancozeb. The selection of fenarimol-resistant isolates resulting from poor control of the resistant subpopulation by the low rate of fenarimol was equivalent whether fenarimol was applied singly or in mixture with mancozeb. Consequently, the use of high DMI rates in mixture with a protective fungicide is expected to delay the build-up of resistant subpopulations by limiting their increase through two separate principles of control. For dodine in mixture with fenarimol, it was found that each mixing partner applied alone selected both fenarimol- and dodine-resistant isolates. This selection pattern was partly explained by the possibility that one of the multiple genes underlying fenarimol and dodine resistance confers resistance to both fungicides, in addition to the selection of double-resistant isolates. Regardless, a mixture of fenarimol with dodine each employed at a low rate controlled both the fenarimol- and the dodine-resistant subpopulation at least as effectively as the individual components at twice their mixture rate, and an accelerated selection of double-resistant isolates was not detected. In commercial orchard trials, mixtures of DMIs with either a protective fungicide or with dodine provided equivalent control even when levels of DMI resistance, dodine resistance, or both were moderately elevated. With the exception of orchards with high levels of DMI or dodine resistance, dodine might be an alternative to protective fungicides as a mixing partner with DMIs.

Several fungicides introduced in the 1980s for the control of tree fruit diseases act as sterol demethylation inhibitors (DMIs; 9,18). Practical resistance to DMI fungicides of *Venturia inaequalis* (Cooke) G. Wint., the causal agent of apple scab, was first documented for an experimental orchard in Nova Scotia, Canada, after DMIs had been tested at the site for more than 10 years (2,3,16). Subsequently, similar resistance to DMIs was identified in a commercial orchard in Michigan (16), and evidence for resistance was provided also for orchards in Europe (19). In anticipation of resistance development of *V. inaequalis* to DMIs, their mixtures with protective fungicides such as mancozeb or captan were suggested as an anti-resistance strategy (25); such mixtures are now commonly used for the control of apple scab with DMI fungicides. Tank-mixing DMIs with protective fungicides also has been recommended to improve the control of fruit infections in a delayed-spray program developed for low-inoculum orchards and with baseline-sensitive populations of *V. inaequalis* (31). The merits of such mixtures in delaying and managing DMI resistance have never been evaluated under orchard conditions. Furthermore, the reliance on purely protective fungicides as components of anti-resistance strategies might not be sustainable, because several of the suitable representatives remain under toxicological scrutiny in various countries.

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Dodine, introduced in the late 1950s as a fungicide for control of apple scab, might serve as an alternative to conventional protectants in mixtures with DMIs. In addition to providing strong protective activity against scab infections, dodine also can be active when applied in pre- and post-symptom modes by preventing production of conidia from established scab lesions (1,30). However, dodine efficacy is often weak in typical after-infection applications (30). In contrast, DMIs are most active in an after-infection mode of application, whereas their protective and anti-sporulant activities are weak (22,26,30). Thus, mixtures of DMIs and dodine might be expected to complement the strength and weakness of each individual component in controlling scab at the various stages of disease development.

The effectiveness of DMIs in mixture with dodine will be influenced by the sensitivity of a given *V. inaequalis* population to both of the components. The first cases of dodine resistance were noted in the late 1960s, after the fungicide had been used extensively for approximately 10 years in scab control programs (7), and resistance became widespread during the 1970s (7,8,20,27,32). A quantitative test for measuring sensitivities of *V. inaequalis* isolates to dodine allowed us to quantify frequencies of dodine-resistant isolates in both baseline populations and populations from orchards with practical resistance to dodine (17). Monitoring of orchards in New York and Michigan revealed that dodine resistance levels (i) largely reflected the dodine use histories at particular sites; (ii) had declined below threshold values in orchards with previous records of dodine resistance after dodine use was suspended for several years; and (iii) could quickly exceed threshold values when dodine was used as a single fungicide at sites where resistance levels were elevated (17).

The development of DMI resistance within populations of *V. inaequalis* in North American apple orchards (2,3,16) signals the need for the development and implementation of effective anti-resistance strategies incorporated into current scab-control programs. The particular nature of resistance development to DMI fungicides has been described variously as directional, quantitative, or polygenic selection (9,11-13,15). In general, baseline populations of fungal pathogens, including *V. inaequalis*, exhibit a broad range of isolate sensitivities to DMIs (3,9,11-16,28); practical resistance develops when selection causes the frequency of the least-sensitive baseline isolates to increase above a threshold value (16). In contrast to other fungicides such as benomyl (12,13), however, the response of isolates resistant to DMIs remains dose dependent (16). Consequently, a theoretical anti-resistance strategy would be to avoid the use of low application rates in order to maximize the control of isolates that would fully resist low doses of a DMI fungicide and, thus, would be selected more rapidly. In this study, the influence of DMI rates and of tank-mixing a DMI with either mancozeb or dodine was evaluated with respect to resistance development and apple-scab control in both experimental and commercial orchard trials.

MATERIALS AND METHODS

Test orchards. Tests were conducted from 1992 to 1994 in an experimental apple orchard (cv. Cortland) in Geneva, New York. This orchard was planted in 1967 and had served as a site for fungicide efficacy testing until 1987. The first DMI fungicides were tested in 1971 and increasingly intense DMI testing continued until 1987. Dodine was tested extensively in this orchard during the 1970s. From 1988 to 1991, the orchard served as a test site for insecticide and acaricides. Apple scab was controlled with DMIs during the four seasons preceding the tests described here.

Tests were also conducted in six commercial orchards in 1996 in cooperation with participating growers. The orchards (cv. McIntosh) were chosen to represent a typical cross-section of DMI use histories (i.e., each had been treated with fenarimol, myclobutanil, or both, predominantly in mixture with either mancozeb or metiram, for at least parts of the previous seven seasons). DMI resistance was not suspected by any of the growers. Each test block was approximately 4 ha in size, and all orchards were located near the south shore of Lake Ontario, three each in the counties of Wayne and Orleans. The two groups of orchards were separated by approximately 150 km.

Application and evaluation of fungicides. In the experimental orchard, fungicide treatments were arranged in a randomized complete block design with three replications. Individual treatment-blocks consisted of two to three trees, and the same trees were assigned to the same treatment each year. Spray solutions were applied dilute (approximately 2,800 liters/ha) with a handgun to the point of runoff. The spray program was designed to target the period from tight cluster through first cover as suggested by Wilcox et al. (31); however, the timings of individual applications were adjusted more closely to specific apple-scab infection periods, with the objective of providing sufficient disease incidence to facilitate the monitoring of isolate sensitivities within each treatment regime.

In 1992, primary infection periods determined as described previously (31) were recorded on 25 April; 1, 17, and 26 May; and 6 and 13 June. Fungicides were applied on 5 and 21 May and 3 June. In 1993, extend the experimentation period in order to obtain sufficient isolate numbers for population profiling. In this year, infection periods were recorded on 22 April; 6, 19, 23, and 31 May; 20 and 29 June; and 14 July; fungicides were applied on 6, 17, and 28 May; 22 June; and 13 July. In 1994, primary infection periods occurred on 28 April; 8, 17, and 25 May; and 14 June; fungicides were applied on 3 and 19 May and 3 and 10 June.

The objective of the experimental orchard trials was to determine the impact of different DMI use strategies on the control of *V. inaequalis* subpopulations either sensitive or resistant to DMIs. Therefore, the evaluation of treatment efficacies was focused on the disease incidence of terminal leaves as the parameter most indicative of disease progression over the primary scab season. In order to allow for lesion development in response to the final treatment, scab incidence (10 leaves each on 10 terminals per tree from the central part of each plot) was assessed 2 weeks after the last fungicide spray was applied. Diseased terminal leaves with actively sporulating lesions were sampled for isolate sensitivity tests at or close to the time disease incidences were evaluated. A total of 40 to 50 isolates from individual and well-dispersed leaves (16,17) were tested for each treatment in each season. In order to minimize significant mixing of the different subpopulations over the course of the experiments, leaves were sampled from the inner parts of respective treatment blocks. The potential for mixing of populations was further reduced by the low-density nature of the orchard (9-m row and 5-m tree spacing).

Growers cooperating in the commercial orchard trials in 1996 were asked to apply a DMI mixed with an ethylenebisdithiocarbamate (EBDC) to half of the test orchard, and a DMI-dodine mixture to the other half on the same dates, using their own application equipment, spray practices, and timing regimes. All growers started their scab-control program during the first week of May, at approximately the tight-cluster stage of blossom bud development as recommended previously for a reduced DMI spray program (31).

Orchards 1 to 3 and 6 received four DMI mixture applications, while orchards 4 and 5 received three and five applications, respectively. The mixtures were applied at intervals ranging from 5 to 15 days, with a mean interval of 11 days. DMIs used were commercial formulations of fenarimol (Rubigan 1EC) applied at a rate of 82 g/ha or of myclobutanil (Nova 40W) applied at a rate of 140 g/ha. EBDCs used as mixture components were mancozeb (Dithane 75DF) or metiram (Polyram 80WP) applied at rates of approximately 2,500 g/ha. Dodine (Syllit 65W) used as the alternative mixture component was applied at a rate of lowed by cover sprays applied to the entire orchard, consisting of captan alone, or captan mixed with sulfur or thiophanate-methyl. Scab incidences were assessed on 10 arbitrarily chosen trees per treatment by examining cluster leaves (25 clusters per tree), fruit (50 fruit per tree), and terminal leaves (10 leaves per terminal, 10 terminals per tree) during 25 June to 29 July.

In order to test the levels of resistance of the respective *V. inaequalis* populations to both DMIs and dodine at the start of the season, growers were asked to leave several trees at the orchard corners unsprayed until the scab incidence reached a level suitable for sampling. Diseased cluster leaves were arbitrarily sampled from these trees on a single date for each orchard during 28 May through 11 July.

Sensitivity tests. Procedures employed for the isolation and propagation of monoconidial isolates of *V. inaequalis* and for testing their sensitivities to fungicides have been described in detail (17,28). Briefly, conidia originating from distinct scab lesions were germinated on water agar amended with antibiotics, and single germinating conidia were transferred to potato dextrose agar (PDA). Fungicide sensitivities of mycelia developing from single conidia were determined by comparing the colony diameters of mycelia developing on PDA or on PDA amended with a fungicide.

Isolate sensitivities were expressed as relative growth (RG) of mycelial colonies at discriminatory doses of the respective fungicides, which were 0.05 $\mu g\ ml^{-1}$ for fenarimol, 0.1 $\mu g\ ml^{-1}$ for myclobutanil, and 0.2 $\mu g\ ml^{-1}$ for dodine (14,16,17). RG was defined as mean colony diameter on PDA amended with the discriminatory dose per colony diameter on unamended PDA $\times$ 100. Fenarimol (technical grade) was obtained from Dow Agrosciences (Indianapolis, IN); myclobutanil (technical grade) from Rohm and Haas (Philadelphia, PA), and dodine (analytical standard) was from Cyanamid (Princeton, NJ). Fungicides used in orchard trials were commercial formulations as specified.

Data analysis. Sensitivities of *V. inaequalis* isolates to DMIs and dodine were analyzed as described previously (16,17). Categorical sensitivity data were compared according to the log linear model of SYSTAT (version 5.2; Systat, Inc., Evanston, IL), with numbers of isolates grouped into the categories sensitive (S) and resistant (R). Isolates with RG values >80 determined for fenarimol were classified DMI-resistant (16), whereas isolates with RG values >90 determined for dodine were classified resistant to that fungicides (17).

Mean RG values were compared with the nonparametric Kolmogorov-Smirnov test of SYSTAT version 5.2. Comparisons of mean RG values were restricted to isolates classified as sensitive to fenarimol or dodine. The objective was to detect any selection of least-sensitive isolates classified as DMI- or dodine-sensitive according to the criteria described previously (16,17).

Additive, synergistic, or antagonistic interactions between mixture components were analyzed by applying the formula $E_{xp} = X + Y - XY/100$ described by Richter (24), with E_{xp} as the percentage of control expected from additive effects of the two components when tested in mixture, and with X and Y as the percentages of control provided by each of the two components independently. Synergism is indicated if the percentage of control observed with the mixture (O_{bs}) is higher than the expected additive effect; antagonism is indicated if the observed control is lower than expected.

RESULTS

Sensitivities of *V. inaequalis* isolates to fenarimol and dodine. The frequency of fenarimol-resistant isolates (RG > 80) sampled from leaves of non-treated trees in 1992 was 7% and significantly higher (P = 0.05) than the frequency of 1.7% determined for typical baseline populations (16); the frequency of dodine-resistant isolates (RG > 90) was 7% and also significantly higher (P = 0.02) than the baseline value (17). By the third year of our tests, resistance levels determined for isolates sampled from non-treated trees were 14% for fenarimol and 8% for dodine. Neither increase was significant (P = 0.3 and 0.9, respectively); therefore, all sensitivity data for isolates sampled from nontreated trees over the three-year period were combined to reflect the orchard population prior to treatments in individual plots. The sensitivity distributions of these isolates were significantly different from the distributions described for baseline populations (Fig. 1). In addition to significantly higher frequencies of resistant isolates, the mean RG value of the fenarimol-sensitive subpopulation had increased from 41 in baseline populations (16) to 53 (P < 0.01); the mean RG value of the dodine-sensitive subpopulation was 55 compared to 41 in baseline populations (P < 0.01; 17).

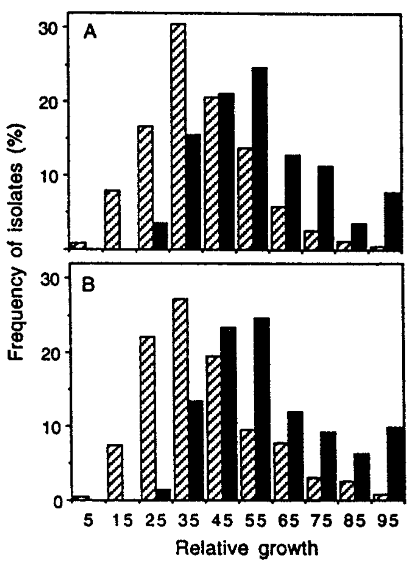

Fig. 1. Distribution of fenarimol and dodine sensitivities of *Venturia inaequalis* populations. **(A)** Fenarimol sensitivity distribution in baseline populations (hatched bars; n = 748) (16) and in the experimental test orchard (closed bars; n = 142). **(B)** Dodine sensitivity distribution in baseline populations (hatched bars; n =

Relative efficacy of apple-scab control. Fenarimol and mancozeb applied alone at half of the rates typically recommended for commercial control of scab (15 and 900 mg/liter, respectively) provided the least control in all three seasons, whereas the combination of the two fungicides was significantly more effective (Table 1). The interactive effects of the two mixture components ($O_{bs} - E_{xp}$ = –5, +9, and +5 for 1992, 1993, and 1994, respectively) suggested that effects were largely additive. Fenarimol alone applied at twice the rate utilized in the mixture provided control equivalent to the half-rate mixture with mancozeb (Table 1). Although incidences of scab on non-treated trees ranged from 23.5 to 85.6% during the three years of testing, levels of control achieved with the various treatments were uniform, with the exceptions of fenarimol applied at the low rate and of dodine (Table 1).

The control of scab achieved with dodine at a rate of 290 mg/liter, which reflects the low range of the rate typically recommended, was similar to the high rate of fenarimol, although seasonal variations were high (Table 1). The mixture of fenarimol and dodine was as effective as the single components applied at twice their mixture rates. Furthermore, the level of control achieved with this mixture was very consistent over the three years of testing (Table 1).

Impact of scab-control tactics on development of resistance to fenarimol and dodine. All treatments significantly increased the frequencies of fenarimol-resistant isolates relative to the population of isolates sampled from non-treated trees (Table 2). Differences among treatments in the frequencies of fenarimol-resistant isolates were not significant (P > 0.32). Mean RG values of fenarimol-sensitive isolates surviving the spray regimes increased significantly only for the low rate of fenarimol applied alone or in mixture with mancozeb (Table 2). Thus, isolates belonging to the least-sensitive part of the DMI-sensitive subpopulation (16) were selected by these treatments but not by the low rate of fenarimol mixed with dodine or the high rate of fenarimol applied singly.

All treatment regimes except the fenarimol-mancozeb mixture resulted in significantly higher frequencies of dodine-resistant isolates (Table 2). The selection of dodine-resistant isolates was significantly more pronounced for the dodine-only treatment than for any other treatment ($P \leq 0.05$), with the exception of fenarimol applied at the high rate (P = 0.14). A higher mean RG value for the dodine-sensitive population was detected only when dodine was applied as the single fungicide (Table 2), indicating that dodine at the rate it was used in our trials also selected isolates belonging to the least-sensitive spectrum of the population normally classified as dodine-sensitive (17).

Differential levels of control achieved for subpopulations sensitive or resistant to fenarimol or dodine. In order to evaluate the significance of differential levels of control achieved for either fungicide-resistant or -sensitive isolates (16), the incidences of apple scab recorded in each of the three years for non-treated trees and for trees subjected to the various treatments (Table 1) and the corresponding frequencies of fenarimol- and dodine-resistant isolates (Table 2) were utilized to separately calculate the percentages of scab control achieved for the sensitive and resistant subpopulations (Table 3).

Table 1. Control of apple scab with single fungicides and fungicide mixtures in experimental orchard trials

Treatment[x]	Rate (mg a.i./liter)	Incidence[w]			Mean control (%)[y]
		1992	1993	1994	
None	…	53.3	23.5	85.6	…
Mancozeb	900	29.0	12.9	35.6	50 (7) a[z]
Fenarimol	15	15.3	14.4	44.5	53 (17) a
Fenarimol	15	11.3	6.0	13.8	79 (5) b
+ Mancozeb	+900	…	…	…	…
Fenarimol	30	14.0	3.9	15.0	80 (5) b
Dodine	290	11.3	1.8	31.0	78 (14) ab
Fenarimol	15	6.7	4.1	13.1	85 (2) b
+ Dodine	+145	…	…	…	…

[w] Mean percentage of terminal leaves infected.
[x] Mancozeb, fenarimol, and dodine were applied as Dithane 75DF, Rubigan 1EC, and Syllit 65 WP, respectively.
[y] Standard deviations are given in parentheses.

Fenarimol applied alone at the low rate provided the least and least-uniform control of the fenarimol-resistant subpopulation (Table 3). Although control was 42% in 1992, no control was provided in 1993 and 1994. Applying fenarimol at twice the rate substantially (P = 0.07) improved control of the resistant population (Table 3). Differences were most pronounced for 1993 and 1994, during which mean control of the fenarimol-resistant subpopulation was 62% versus 0% for the high and low fenarimol rate, respectively.

Control of the resistant subpopulation was also substantially (P = 0.07) improved when fenarimol at the low rate was applied in mixture with mancozeb (Table 3). However, this improved level of control was due to the indiscriminate contribution provided by mancozeb. Eliminating this mancozeb contribution revealed that control of the fenarimol-resistant subpopulation provided by the low rate of fenarimol was equally poor (P = 0.87) whether the fungicide was applied alone or in mixture with mancozeb (Table 3). Likewise, the control of the sensitive population provided by the low rate of fenarimol contained in the mixture or applied alone was equivalent (P = 0.59), indicating that the relative contribution of mancozeb was additive for both the DMI-resistant and -sensitive subpopulations. The most effective and most consistent control of the fenarimol-resistant subpopulation was achieved with the low-rate mixture of fenarimol and dodine (Table 3).

Because selection of dodine-resistant isolates was most pronounced for dodine applied singly (Table 2), poor control of the dodine-resistant subpopulation was also expected to be most pronounced for this treatment. The data reflected this expectation, but the differential control of the dodine-sensitive and -resistant subpopulations were of low statistical significance (Table 3) due to the variable control of the dodine-resistant population provided by dodine applied alone (percentages of control were 43, 78, and 3 in 1992, 1993, and 1994, respectively). The best and most consistent control of the dodine-resistant subpopulation was achieved with the dodine plus fenarimol mixture (Table 3), very similar to the consistently high level of control of the fenarimol-resistant population provided by the same mixture.

Although differences were not always of high statistical significance due to the sometimes large variations over the three test seasons, control of the fenarimol-resistant subpopulation was consistently lower for all treatments, including dodine applied alone (Table 3). Conversely, the inferior control of the dodine-resistant population with fenarimol also was observed (Table 3).

Interdependence of DMI and dodine resistance. The possibility that the selection of fenarimol-resistant isolates by dodine and vice versa (Tables 2 and 3) might be explained by the selection of isolates double-resistant to both inhibitors

Table 2. Levels of resistance of *Venturia inaequalis* populations controlled with fenarimol, dodine or fungicide mixtures

			Treatments[u]					
Fungicide	**Isolate values**	**Check[v]**	**Fenarimol (15)**	**Fenarimol (30)**	**Fenarimol (15) + mancozeb (900)**	**Dodine (290)**	**Fenarimol (15) + dodine (145)**
Fenarimol	n	142	133	149	150	150	150
	R[w]	11.3	22.6	26.1	26.0	23.3	21.3
	P^x	…	0.01	<0.01	<0.01	<0.01	0.02
	Mean RG (sen)[y]	52.7	55.4	52.5	55.8	53.2	53.5
	P^z	…	0.04	0.38	0.03	0.40	0.25
Dodine	n	142	128	149	150	150	150
	R[w]	9.9	17.2	19.5	10.7	26.7	16.7
	P^x	…	0.05	0.01	0.67	<0.01	0.05
	Mean RG (sen)[y]	55.0	55.7	55.0	52.8	59.7	55.8
	P^z	…	0.71	0.98	0.60	0.01	0.63

[u] Rates of fungicides (g a.i./1,000 liters) are given in parentheses.
[v] Isolates collected from nontreated trees.
[w] Frequencies of isolates (%) classified as resistant (R) to fenarimol (relative growth [RG] > 80) or dodine (RG > 90).
[x] Comparison of counts of isolates classified as sensitive (S) or R obtained from nontreated trees with respective counts for isolates obtained from trees treated as specified (log linear model).
[y] Mean RG values of isolates sensitive to fenarimol (RG ≤ 80) or dodine (RG ≤ 90).
[z] Comparison of mean RG values determined for the sensitive population of isolates retrieved from nontreated trees with respective means for isolates retrieved from trees treated as specified (Kolmogorov-Smirnov test).

Table 3. Control of *Venturia inaequalis* subpopulations sensitive (S) or resistant (R) to fenarimol or dodine

		Mean control (%)[v]					
		Fenarimol			Dodine		
Treatment	**Rate (mg a.i./liter)**	**S**	**R**	**P^w**	**S**	**R**	**P^w**
Fenarimol	15	59 (14)[x]	14 (24)	0.05	57 (16)	20 (27)	0.13
Fenarimol	30	83 (4)	54 (14)	0.10	82 (5)	60 (10)	0.02
Fenarimol	15	…	…	…	…	…	…
+ Mancozeb (mixture)[y]	900	83 (4)	52 (11)	0.01	79 (5)	78 (4)	0.74
Fenarimol	15	…	…	…	…	…	…
+ Mancozeb (fenarimol)[z]	900	64 (4)	17 (8)	0.01	62 (4)	27 (9)	0.004
Dodine	290	82 (12)	56 (30)	0.23	83 (12)	39 (38)	0.14
Fenarimol	15	…	…	…	…	…	…
+ Dodine	145	87 (7)	72 (5)	0.02	86 (2)	74 (5)	0.01

[v] The levels of control (relative to the untreated check) achieved for sensitive and resistant subpopulations were calculated from disease incidences recorded for the various treatments in each year and averaged frequencies of sensitive or resistant isolates determined for isolates obtained from the respective treatments.
[w] Significance of differences between mean percentages of control achieved for sensitive and resistant subpopulations (analysis of variance).
[x] Standard deviations are given in parentheses.
[y] Calculated from the disease control and resistance frequency for trees treated with the mixture.

was analyzed for all isolate sensitivities determined in 1993 and 1994. In these two seasons, sensitivities to myclobutanil were tested in addition to fenarimol in order to provide comparative data for a second DMI fungicide.

The fenarimol-sensitive subpopulation contained a significantly lower frequency of dodine-resistant isolates than the fenarimol-resistant subpopulation (Table 4). Although of slightly lower statistical significance, the same relationship applied to myclobutanil (Table 4). The result of this analysis indicated that resistance of *V. inaequalis* to DMIs and dodine was not an entirely independent trait, as would have been expected if double resistance arose from a random distribution of distinctly different resistance genes within a population. Applying the mixture of fenarimol and dodine could have been expected to accelerate the selection of double-resistant isolates. This expectation was not confirmed. The mean frequency of fenarimol-resistant isolates obtained from treatments with the mixture was 18.6% and that of dodine resistance was 12.7%. If fenarimol and dodine resistance were independent traits, the expected frequency of double-resistant isolates would be 2.4%. The frequency determined was 2.7% and, thus, not different from the expected frequency considering the sample size of 150 isolates tested.

In addition, the comparison of fenarimol and myclobutanil sensitivities (Table 4) confirmed the high degree of cross-resistance described before (14,16). Although myclobutanil had not been applied in the orchard, frequencies of myclobutanil-resistant isolates were as high as fenarimol frequencies (Table 4).

Evaluation of DMI mixtures with mancozeb and dodine under commercial orchard conditions. Frequencies of resistance to fenarimol and myclobutanil for isolates retrieved from nontreated trees in six commercial orchards ranged from baseline to significantly higher than baseline (Table 5). However, all orchard populations were significantly ($P < 0.01$) less resistant than the threshold of practical resistance determined previously (16). For dodine, resistance was baseline at one of the orchards; at all other sites, resistance levels were significantly higher than baseline (Table 5). With the exception of orchard 4, all other levels of dodine resistance were also significantly ($P < 0.01$) lower than the threshold described for dodine (17; Table 5).

When a DMI was mixed with either mancozeb or dodine, scab control was equivalent or significantly higher with dodine as the mixing partner (Table 6). Scab control was not compromised at the two sites with elevated frequencies of isolates resistant to both fenarimol and dodine (orchards 5 and 6, Table 6). The different levels of control observed among orchards appeared to be more closely related to disease management practices rather than to respective levels of resistance at these sites. For example, the poor control of scab obtained in orchard 4 with myclobutanil employed as the DMI was associated with a *V. inaequalis* population whose frequency of myclobutanil-resistant isolates was not different from baseline (Table 5). Although the frequency of dodine-resistant isolates was relatively high in this orchard (Table 5), scab control was equally poor whether mancozeb or dodine was used as the mixing partner, providing further evidence that poor control of apple-scab development was due to inadequate management practices rather than fungicide resistance.

DISCUSSION

Strategies for delaying and managing fungicide resistance have two major goals: (i) slowing the rates at which resistant subpopulations are selected to sizes large enough to cause unacceptable levels of disease in the presence of respective fungicides, and (ii) preventing resistant subpopulations from compromising commercially acceptable disease control once resistant phenotypes have been selected to high frequencies. Both aspects were investigated in this study.

For fungicides characterized by a broad range of isolate sensitivities detected in baseline populations, as exemplified by both the DMIs (16) and dodine (17), the definition of isolates qualifying as resistant under practical conditions is central to efforts for monitoring resistance development and evaluating programs for its delay and management. Particular isolate sensitivities of *V. inaequalis* were defined resistant to DMIs or dodine, because such isolates had increased from low yet detectable baseline levels to high frequencies at sites with unsatisfactory control of apple

Table 4. Relationship between resistance of *Venturia inaequalis* isolates to two sterol demethylation inhibitor (DMI) fungicides and to dodine

	Fenarimol[x]		Myclobutanil	
	S	R	S	R
Dodine R[y]	16.3	24.8	16.6	23.5
n	472	165	477	162
P^z	0.02		0.06	

[x] S = sensitive, R = resistant.
[y] Frequency (%) of dodine-resistant isolates (relative growth [RG] > 90) in populations either S or R (RG > 80) to the DMIs fenarimol or myclobutanil.
[z] Comparison of counts of dodine-resistant isolates classified as S or R to the DMIs fenarimol or myclobutanil (log-linear model).

Table 5. Sensitivities to sterol demethylation inhibitor fungicides and dodine of *Venturia inaequalis* populations in six commercial New York apple orchards employed in 1996 trials

Fungicides	Baseline[x]	Threshold[x]	Orchards 1	2	3	4	5	6
Fenarimol								
n	748	104	50	50	50	50	50	50
R[y]	1.7	40	2	4	4	4	10	12
P (baseline)[z]	…	…	0.89	0.32	0.32	0.32	<0.01	<0.01
Myclobutanil								
n	627	104	50	50	50	50	50	50
R[y]	2.2	43	2	4	6	2	8	14
P (baseline)[z]	…	…	0.91	0.47	0.16	0.91	0.04	<0.01
Dodine								
n	232	174	50	50	50	50	50	50
R[y]	0.9	41	4	10	8	48	12	10
P (baseline)[z]	…	…	0.14	<0.01	0.03	<0.01	<0.01	<0.01

[x] Baseline and threshold frequencies (%) of resistant isolates are from Köller et al. (16) for fenarimol and myclobutanil, and from Köller et al. (17) for dodine.
[y] Frequencies of isolates (%) in the category resistant (R; relative growth [RG] > 80 for fenarimol and myclobutanil, RG > 90 for dodine) (16,17).

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