Evidence for the Predisposition of Fungicide-Resistant Isolates of *Venturia inaequalis* to a Preferential Selection for Resistance to Other Fungicides

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ABSTRACT

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In the United States, populations of the apple scab pathogen *Venturia inaequalis* have progressed through three consecutive rounds of fungicide resistance development, first to dodine, then to the benzimidazoles, and most recently to the sterol demethylation inhibitors (DMIs). Analysis of extensive monitoring data have to date provided no indication of detectable cross-resistance or partial cross-resistance of *V. inaequalis* populations to the three unrelated classes of fungicides prior to the selection of resistant subpopulations. However, in this study, resistance to both benomyl

and DMIs developed to significantly higher frequencies within the previously established dodine-resistant population than in the population sensitive to dodine. Accelerated selection of phenotypes double resistant to dodine and the DMI fenarimol was apparent over the course of distinct seasons of apple scab management with either dodine or fenarimol. The data provide evidence for an accelerated speed of resistance development among phenotypes of *V. inaequalis* already resistant to an unrelated fungicide. This finding represents a departure from the previous model, which assumed entirely independent rounds of resistance developments. The data indicate that phenotypes of *V. inaequalis* might not only be selected for the trait of fungicide resistance but also for traits allowing a more flexible response to changes in the environment where they compete.

Scab caused by *Venturia inaequalis* (Cooke) G. Winter is recognized as the economically most important disease of apples (23). Fungicides remain the primary tool for managing this disease, and due to polycyclic nature, repeated applications are required over the course of a single season (23). This intensive use of fungicides has been responsible for the more or less rapid development of resistance to several classes of specific fungicides (7,9,10,20,21).

In the United States, populations of *V. inaequalis* have progressed through three consecutive rounds of resistance development. Practical resistance was first documented for dodine in the late 1960s and early 1970s, after the fungicide had been used extensively for approximately 10 years (7,9,21). In response, dodine was replaced by benzimidazole fungicides followed by the development of practical benzimidazole resistance after 2 to 4 years of widespread use (7,9). The class of sterol demethylation inhibitors (DMIs) was introduced in the late 1980s, and practical resistance of *V. inaequalis* populations to DMIs under commercial conditions of apple production was first documented in 1995 (20). In 1999, the class of strobilurin-related fungicides such as kresoxim-methyl and trifloxystrobin was introduced as new tools for the management of scab and other apple diseases (24,34). Risk assessment studies (28,29,35,36) have indicated that resistance of *V. inaequalis* populations to strobilurin-related fungicides will likely develop, which would constitute a fourth round of resistance.

Repeated replacement of one fungicide class by another in response to practical resistance mandates that the various classes of fungicides deployed consecutively lack cross-resistance. For *V. inaequalis*, such a lack of cross-resistance was apparent for dodine, the benzimidazoles, and the DMIs. Initial levels of scab

control achieved with benzimidazoles in dodine-resistant orchards were reported to be high (7,9), and DMIs performed well in orchards located within a region known for widespread dodine and benzimidazole resistance (32). The apparent lack of cross-resistance among the different classes of fungicides indicates that the selection of resistant subpopulations proceeded independently from each other.

However, we recently documented that phenotypes of *V. inaequalis* resistant to dodine were not only selected by dodine but also during treatments with DMIs. Likewise, DMI-resistant phenotypes were also selected by dodine, indicating that the mechanisms of resistance to these fungicides might not be entirely independent (18,19). In this study, evidence is presented that *V. inaequalis* phenotypes selected for dodine or benomyl resistance remained baseline sensitive to other classes of fungicides prior to a new round of selection for resistance, but that phenotypes already resistant to dodine were prone to accelerated adaptations to other fungicides, revealing for the first time that the speed of developing resistance to one class of fungicides may be accelerated in case of preexisting resistance to another class of unrelated fungicides.

MATERIALS AND METHODS

Origin of isolates and sensitivity tests. Ten commercial orchards in New York were sampled in 1991 as described previously (20,21). Isolates from these orchards were used to assess the impact of dodine resistance on the development of benomyl resistance. A total of 40 commercial orchards in Michigan, New Hampshire, New York, North Carolina, Pennsylvania, and Virginia were sampled from 1995 to 1999. Isolates from these orchards were obtained in June from first scab lesions developing on trees not sprayed with fungicides as described previously (20,21). Respective isolates were used to assess the impact of dodine resistance on the selection of DMI-resistant subpopulations.

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Apple trees were sampled in an experimental orchard in 1992, 1993, and 1994 as described elsewhere in detail (18,19). For the data analyzed in the present study, diseased leaves were collected from nontreated trees and from trees treated with either dodine (Syllit 65W; 290 mg a.i. liter⁻¹) or the DMI fenarimol (Rubigan 1EC; 30 mg a.i. liter⁻¹), both applied at rates recommended for the commercial control of apple scab (18,19). Trees were sprayed three, five, and four times in 1992, 1993, and 1994, respectively, before diseased leaves were collected in late June or early July (18,19). All 40 to 50 isolates analyzed for each orchard or for each experimental treatment originated from single conidia retrieved from scab lesions developing on separate leaves sampled from several trees (18–21).

Fenarimol (technical grade) was obtained from Dow Agrosciences (Indianapolis), dodine (analytical standard) from Cyanamid (Princeton, NJ), and benomyl (technical grade) from DuPont (Wilmington, DE). Sensitivity tests with monoconidial isolates of *V. inaequalis* were done according to the procedures described previously (17–21). In brief, mycelia of isolates were tested for sensitivities to dodine, to the representative DMI fenarimol, and to the benzimidazole benomyl at discriminatory doses of 0.2 µg ml⁻¹ (21), 0.05 µg ml⁻¹ (20), and 0.5 µg ml⁻¹ (12), respectively. Sensitivities of isolates were expressed as relative growth (RG; mean diameter of mycelial colonies developing on fungicide-amended medium per diameter on fungicide-free medium × 100). Isolates were rated resistant to dodine, fenarimol, or benomyl when their RG values were >90 (21), >80 (20), or >20 (12), respectively.

Data analysis. Sensitivity data were retrieved and analyzed as described previously (18–21). In brief, populations of isolates distinguished by distinct patterns of sensitivities to dodine, benomyl, and fenarimol were assembled to composite populations. Mean RG values of isolates categorized as sensitive to dodine or fenarimol were compared by *t* test analysis, because selection for resistances to either dodine or fenarimol was also reflected by changes of mean sensitivities within subpopulations categorized as sensitive, in addition to changes in frequencies of resistant isolates (18,19). Independent *t* test analysis was supported by SYSTAT (version 5.2; Systat Inc., Evanston, IL). Frequencies of isolates resistant to dodine, benomyl, or fenarimol were compared by fitting log-linear models with counts of isolates grouped into the categories sensitive and resistant to the fungicides analyzed. The model supported by SYSTAT assumes homogeneity of isolate sensitivities in subpopulations sensitive or resistant to an unrelated fungicide, with nonhomogeneity indicating interdependence of isolate sensitivities to an unrelated fungicide.

RESULTS

Impact of dodine resistance on the development of benomyl resistance. Out of 10 commercial orchards sampled throughout New York in 1991, *V. inaequalis* populations retrieved from seven of the orchards exhibited significantly ($P < 0.01$) elevated levels of dodine resistance based on log-linear comparisons of resistance frequencies with the frequency typical for baseline populations (21). The respective composite population assembled by combining all isolate sensitivities from the seven orchards (Fig. 1) departed significantly ($P < 0.01$) from baseline populations with respect to both the mean RG value of dodine-sensitive isolates (RG = 44 versus 40 in baseline populations) and the frequency of dodine-resistant isolates (15 versus 0.9% in baseline populations). Therefore, the composite of isolates represents a population of *V. inaequalis* with elevated levels of dodine-resistant phenotypes established through selection. In all seven orchards comprising the respective composite population, apple scab had also been managed with benzimidazole fungicides, resulting in the selection of benomyl-resistant isolates of *V. inaequalis* to a frequency of 44% (Fig. 2), in addition to the elevated frequency of dodine-resistant isolates (Fig. 1).

For independent fungicides such as dodine and benomyl with their distinctly different modes of action (1,3), it was expected that the initial baseline frequencies of benomyl-resistant and, thus, selectable mutants would be the same in both the dodine-sensitive and dodine-resistant subpopulations. The speed of selection and, consequently, the frequencies of benomyl-resistant mutants established should be independent from the dodine sensitivities of respective mutants. This expectation was contradicted in the analysis of respective sensitivity distributions established for the composite population (Table 1). Within the dodine-resistant subpopulation (RG > 90), the frequency of benomyl-resistant isolates was nearly twice as high as in the subpopulation fully sensitive (RG = 0 to 50) to dodine (Table 1). This difference was highly significant ($P < 0.001$). The frequency of benomyl-resistant isolates in the dodine-resistant subpopulation was also significantly higher than for the combined composite population (Table 1). Respective differences were of low significance for both the subpopulation reflecting isolates fully sensitive to dodine (RG = 0 to 50) and the subpopulation less sensitive (RG = 51 to 90) than the baseline mean of RG = 40 (21) (Table 1). The results indicated that isolates qualifying as resistant to dodine (21) had been subjected to an accelerated selection toward benomyl resistance.

Fig. 1. Distributions of dodine sensitivities in a composite population of *Venturia inaequalis* sampled from seven commercial orchards in New York distinguished by an elevated level of dodine resistance (closed bars, $n = 278$) versus a composite population reflecting baseline sensitivities (hatched bars; $n = 232$). Baseline data are from Köller et al. (21). Isolate sensitivities are expressed as relative growth at a discriminatory dodine dose of 0.2 µg ml⁻¹.

Fig. 2. Distributions of benomyl sensitivities in a composite population of *Venturia inaequalis* sampled from seven commercial orchards in New York distinguished by an elevated level of dodine resistance ($n = 278$). Isolate sensitivities are expressed as relative growth at a discriminatory benomyl dose of 0.5 µg ml⁻¹.

A preferential selection of isolates exhibiting resistance to both dodine and benomyl was confirmed in the complementary analysis of dodine sensitivity distributions within the benomyl-sensitive and -resistant subpopulations of the same composite population (Fig. 3). Most pronounced was the difference in frequencies of dodine-resistant isolates ($RG > 90$), with 9.6% detected in the benomyl-sensitive versus 22.1% detected in the benomyl-resistant subpopulation ($P = 0.004$).

In contrast with the composite population derived from the seven New York orchards with significant shifts toward both dodine and benomyl resistance, there was little or no prior history of dodine use in the remaining three New York orchards sampled in 1991. Consequently, the dodine sensitivity of the respective composite population was not different from baseline levels (Table 2). However, in the same orchards, scab had been controlled with benzimidazoles such as benomyl, and benomyl-resistant phenotypes had been selected to a frequency of 25% (Table 2). Under these conditions of single resistance to benomyl, dodine sensitivity distributions were not significantly different within the benomyl-sensitive versus the benomyl-resistant subpopulation (Table 2), confirming a lack of detectable interrelation between benomyl- and dodine-resistant phenotypes in orchards baseline sensitive to dodine.

Impact of dodine and benomyl resistance on isolate sensitivities to DMIs under baseline conditions. At the time the seven New York orchards with elevated levels of both dodine and

benomyl resistance (Table 1) were sampled in 1991, DMI fungicides such as fenarimol and myclobutanil had not or rarely been used in scab management (20,21). For the composite population, the mean sensitivity of DMI-sensitive isolates was only slightly yet significantly elevated from a typical baseline population, whereas the frequency of DMI-resistant isolates had remained at a baseline level (Table 3). Under such conditions of only slight responses of populations to DMI treatments, the status of isolate sensitivities to both dodine and benomyl had no significant impact on the distribution of DMI sensitivities (Table 3). The analysis confirmed the lack of detectable cross-resistance between the DMI fenarimol and both dodine and benomyl prior to the selection of DMI-resistant subpopulations to frequencies significantly departing from baseline levels.

Impact of dodine resistance on the selection of DMI-resistant subpopulations. DMI fungicides such as fenarimol and myclobutanil were increasingly employed in the management of apple scab following their introduction in 1987 (10), and the first control failure under commercial orchard conditions due to the selection of a DMI-resistant subpopulation was recorded in 1995 (21). Since then, extensive monitoring efforts were dedicated to discerning the status of DMI sensitivities of *V. inaequalis* populations in commercial apple orchards. Respective tests of isolate sensitivities included dodine in addition to the DMI fenarimol, which allowed the analysis of potential interrelationships between these two unrelated fungicides.

Out of a total of 40 orchard populations tested from 1995 to 1999 and sampled from different regions of the United States, nine exhibited frequencies of resistant isolates $>15\%$ for both dodine and fenarimol, which were significantly ($P < 0.01$) elevated from respective baseline levels on both mean RG values of sensitive isolates and frequencies of resistant isolates (20,21). Respective populations of *V. inaequalis* had presumably experienced shifts toward dodine resistance first, followed by shifts toward DMI resistance. Isolate sensitivity data retrieved from these nine orchards were combined to a composite population in order to analyze the impact of dodine resistance on the selection of DMI-resistant subpopulations.

For the composite population, mean RG values of DMI-sensitive isolates for subpopulations distinguished by declining sensitivities to dodine were not different from the composite population (Table 4). However, the frequencies of fenarimol-resistant isolates established in the populations fully sensitive ($RG = 0$ to 50) or resistant ($RG > 91$) to dodine were significantly lower and higher, respectively, than for the entire composite population (Table 4). Subpopulations exhibiting intermediate ranges of dodine sensitivities reflected frequencies of fenarimol resistance typical for the entire composite population (Table 4). Similar to the relationship between benomyl and dodine resistance described previously

TABLE 1. Status of benomyl sensitivities of *Venturia inaequalis* isolates in a composite population with significant shifts toward both dodine and benomyl resistance^a

Population	n	Benomyl sensitivity (% isolates)	
		Sensitive ^b	Resistant ^c
Composite	278	56.1	43.9
Dodine (0 to 50) ^d	162	64.2	35.8 (0.10)
Dodine (51 to 90) ^d	74	50.0	50.0 (0.35)
Dodine (>90) ^d	42	35.8	64.2 (0.01)

^a The composite population was compiled from sensitivities of isolates collected from seven New York orchards sampled in 1991.

^b Relative growth (RG) = 0 at $0.5 \mu\text{g ml}^{-1}$ of benomyl.

^c $RG > 20$ at $0.5 \mu\text{g ml}^{-1}$ of benomyl. P values given in parentheses reflect comparisons of counts of benomyl-resistant isolates in the subpopulations with respective counts in the composite population (log-linear model).

^d Subpopulations with different dodine sensitivities ranging from fully sensitive ($RG = 0$ to 50) to resistant ($RG > 90$). Ranges of RG values given in parentheses.

Fig. 3. Distributions of dodine sensitivities of *Venturia inaequalis* subpopulations sensitive (closed bars, $n = 141$) or resistant (hatched bars; $n = 95$) to benomyl. Isolate sensitivities are expressed as relative growth at a discriminatory dodine dose of $0.2 \mu\text{g ml}^{-1}$. The two subpopulations were derived from a composite population ($n = 278$) of *V. inaequalis* sampled from seven commercial orchards in New York with elevated levels of dodine and benomyl resistance.

TABLE 2. Status of dodine sensitivities of *Venturia inaequalis* isolates in a composite population with significant shifts toward benomyl resistance but baseline sensitivities to dodine^a

Population	n	Dodine sensitivity	
		Mean RG_S^b	F_R^c
Composite	137	42.0	2.2
Benomyl sensitive	103	41.2 (0.69)	1.9 (0.89)
Benomyl resistant	34	44.5 (0.47)	2.9 (0.80)
Baseline ^d	232	40.1 (0.27)	0.9 (0.29)

^a The composite population was compiled from sensitivities of isolates collected from three New York orchards sampled in 1991.

^b Mean relative growth (RG) values of dodine-sensitive ($RG = 0$ to 90) isolates. P values given in parentheses reflect the comparisons of mean RG values with the composite population (t test).

^c Frequencies (%) of dodine-resistant ($RG > 90$) isolates. P values given in parentheses reflect comparisons of counts of dodine-resistant isolates with the respective count in the composite population (log-linear model).

^d Baseline data obtained from Köller et al. (21).

(Table 1), the frequency of fenarimol-resistant phenotypes established in the dodine-resistant subpopulation was approximately twice as high ($P < 0.001$) as in the dodine-sensitive subpopulation (Table 4).

Impact of resistance to dodine and DMIs on dynamics of isolate selection. The above data indicate a predisposition of dodine-resistant isolates to accelerated selection by both the benzimidazole benomyl (Table 1) and the DMI fenarimol (Table 4). However, these findings were derived from monitoring data retrieved from orchard populations that had been subjected to selection over numerous growing seasons and through variable use patterns of the respective fungicides. Trials conducted over a period of 3 years (18,19) allowed us to evaluate, for a single orchard population and under controlled experimental conditions, the pressure exerted by multiple applications of either dodine or fenarimol on to the selection of isolates resistant to only one or both of the fungicides.

As summarized in Table 5, control of apple scab with dodine significantly increased the frequency of dodine-resistant isolates. Likewise, treatments with fenarimol had significant impact on the selection of DMI-resistant isolates. In both cases, frequencies of isolates exhibiting the opposite pattern of sensitivities (sensitive to the applied fungicide but resistant to the other) were also elevated, but those differences were not significant (Table 5). For both fungicides, the most profound and significant increases in frequencies were observed for isolates exhibiting double resistance to both dodine and fenarimol (Table 5), indicating that the speed of selection by dodine or fenarimol was accelerated for isolates already resistant to the opposite fungicide.

DISCUSSION

Although resistance of plant pathogenic fungi to protective fungicides with nonspecific modes of action has not become a limitation to the sustained control of plant diseases, development of resistance to site-specific inhibitors has curtailed the initial utility of almost all modern classes of fungicides (13,16,31). As documented in numerous studies (7,9,10,20,21), populations of *V. inaequalis* developed resistance consecutively to dodine, benzimidazoles, and DMI fungicides. Thus, the organism represents a unique model for studies aimed at assessing the impact of multiple rounds of population responses toward fungicide resistance.

The most widely accepted model of resistance development implies that fungicide-resistant phenotypes originating from spon-

aneous mutations preexist at a low frequency prior to the introduction of a given fungicide (33). Selection of such preexisting resistant phenotypes will occur with repeated fungicide applications until their frequencies reach the status of practical field resistance (13,20,21). Previous experiences with fungicide resistance implied that mechanisms of resistance were specific to a particular fungicide class, thereby allowing practical resistance to one class of fungicides to be counteracted by the introduction of a new class.

In the case of *V. inaequalis*, experience with the successive introductions of new fungicides gave no indications of a departure from the model (7,9,21,32). Resistance developed first to dodine, a highly selective fungicide primarily used for the control of apple scab (7,9,21). Although mechanistic details of dodine resistance have not been elucidated, resistance development progressed through a pattern typical for polygenic resistance, with resistant phenotypes comprising the least sensitive part of a continuous distribution of isolate sensitivities in baseline populations (21). Prohibitively high levels of practical dodine resistance were counteracted by the introduction of the benzimidazole fungicides, leading to a second round of resistance development (7,9). In contrast to dodine, the mechanism of benzimidazole resistance is exceptionally well understood. The inhibitors bind to fungal β -tubulin and block tubulin polymerization, and high degrees of resistance are caused by mutational amino acid exchanges within the β -tubulin site responsible for benzimidazole binding (3,12). The benzimidazoles were subsequently replaced by the class of DMI fungicides. Resistance to DMIs developed, again, in a pattern typical for polygenic resistance (20). Although the mode of action of DMI fungicides acting as lanosterol demethylation inhibitors is well understood (14,15), a comprehensive characterization of the multiple mechanisms involved in DMI resistance is only emerging at present (4–6,11,25,30).

The data presented in this study reveal, to our knowledge for the first time, strong evidence for an unexpected effect of resistance established to one fungicide on the preferential selection of already resistant phenotypes by another and independent class of fungicides. Specifically, both benomyl- and DMI-resistant phenotypes of *V. inaequalis* were selected to significantly higher frequencies within the dodine-resistant subpopulations selected during the initial round of resistance development. The results suggest that resistance to one class of fungicides can accelerate the speed by which mutants resistant to another yet independent class of fungicides will be selected. Although the data provided

TABLE 3. Status of fenarimol sensitivities of *Venturia inaequalis* isolates in a composite population with significant shifts toward both dodine and benomyl resistance^a

Population	n	Fenarimol sensitivity	
		Mean RG _S ^b	F _R ^c
Composite	278	42.0	2.5
Dodine (0 to 50) ^d	162	42.0 (0.96)	1.2 (0.37)
Dodine (51 to 90) ^d	74	43.5 (0.50)	5.4 (0.19)
Dodine (>90) ^d	42	40.5 (0.32)	2.3 (0.97)
Benomyl sensitive	156	42.7 (0.67)	2.6 (0.95)
Benomyl resistant	122	41.2 (0.60)	2.5 (0.99)
Baseline ^e	748	39.8 (0.04)	1.7 (0.45)

^a The composite population was compiled from sensitivities of isolates collected from seven New York orchards sampled in 1991.

^b Mean relative growth (RG) values of fenarimol-sensitive (RG = 0 to 80) isolates. *P* values given in parentheses reflect the comparisons of mean RG values with the combined population (*t* test).

^c Frequencies (%) of fenarimol-resistant (RG > 80) isolates. *P* values given in parentheses reflect the comparisons of counts of dodine-resistant isolates with the respective count in the composite population (log-linear model).

^d Subpopulations with different dodine sensitivities ranging from fully sensitive (RG = 0 to 50) to resistant (RG > 90). Ranges of RG values are given in parentheses.

^e Baseline data are from Köller et al. (20).

TABLE 4. Status of fenarimol sensitivities of *Venturia inaequalis* isolates in a composite population with significant shifts toward both dodine and fenarimol resistance^a

Population	n	Fenarimol sensitivity	
		Mean RG _S ^b	F _R ^c
Composite	447	57.9	30.9
Dodine (0 to 50) ^d	104	57.0 (0.62)	19.2 (0.02)
Dodine (51 to 70) ^d	155	60.3 (0.12)	29.7 (0.78)
Dodine (71 to 90) ^d	102	55.9 (0.32)	33.3 (0.63)
Dodine (>90) ^d	86	58.2 (0.68)	44.2 (0.02)

^a The composite population was compiled from sensitivities of isolates collected from nine orchards in Michigan, New Hampshire, New York, North Carolina, Pennsylvania, and Virginia, sampled in 1995 and 1999. Frequencies of resistance to dodine and fenarimol were both >15% for each of the orchard populations.

^b Mean relative growth (RG) values of fenarimol-sensitive (RG = 0 to 80) isolates. *P* values given in parentheses reflect the comparisons of mean RG values with the composite population (*t* test).

^c Frequencies (%) of fenarimol-resistant (RG > 80) isolates. *P* values given in parentheses reflect the comparisons of counts of dodine-resistant isolates respective counts in the combined population (log-linear model).

^d Subpopulations with different dodine sensitivities ranging from fully sensitive (RG = 0 to 50) to resistant (RG > 90). Ranges of RG values are given in parentheses.

document predominantly a predisposition of dodine-resistant phenotypes to preferential selection, this predisposition was observed for the strikingly different fungicides benomyl and fenarimol with their principally different modes of action and different patterns of resistance development. It is, thus, doubtful that the predisposition phenomenon documented here is restricted to dodine.

It might be feasible to speculate that one of the several mechanisms responsible for resistance to one class of fungicides such as dodine could also confer resistance to another class with a different mode of action. A membrane transport system responsible for the active exclusion of unrelated fungicides from fungal cells has been described as a potential mechanism conferring such multifungicide resistance (5,6). If such a mechanism of partial cross-resistance among otherwise independent fungicide classes would reflect a major trait of *V. inaequalis* populations, the frequency of isolates carrying this trait should be expected to be increased in subpopulations selected through an initial round of resistance. Such frequency increases would, thus, have detectable impact on the baseline sensitivities to other classes of fungicides prior to their deployment. Because sensitivities of *V. inaequalis* isolates to dodine or the DMI fenarimol were not different for subpopulations preselected for resistance to benomyl or dodine, respectively, the data presented here gave no indication for increased frequencies of phenotypes expressing multifungicide resistance.

The predisposition of fungicide-resistant isolates to preferential selection for double-resistant isolates might rather indicate that fungicides not only select for the trait of resistance to a particular fungicide but also for the trait of increased genetic plasticity, allowing resistant phenotypes to mutationally adapt more rapidly to new conditions within the environment they compete in. Such increased adaptability was apparent from the results presented here for the preferential selection of *V. inaequalis* isolates resistant to both dodine and the DMIs during an orchard trial under controlled and uniform conditions of selection pressure exerted by either dodine or fenarimol. For bacteria, the speed of spontaneous mutations toward antibiotic resistance is substantially elevated in hypermutable (mutator) strains (22,26). One of the causes for this hypermutability has been related to alterations in various DNA repair mechanisms (22,26,27). Very similar mutator mechanisms have been described to be involved in cancer development (27) and were also highly conserved in *Saccharomyces cerevisiae* (2,8). Whether the phenomenon of hypermutability reported in bacteria (22,26,27) and yeast (2,8) is involved in the predisposition of fungicide-resistant phenotypes of *V. inaequalis* to accelerated selection by other fungicides, as documented here and to our knowledge for the first time, remains unknown at present.

According to the data presented in this study, the apparent speed of selection of *V. inaequalis* phenotypes resistant to dodine was

accelerated by a factor of approximately two for both benomyl and the DMI fenarimol. Whether development of practical resistance to new fungicides will be accelerated accordingly in populations already resistant to a fungicide introduced earlier versus populations not yet resistant to a fungicide remains unanswered. Regardless of this uncertainty, antiresistance strategies aimed at slowing the speed of resistance development may have to take the potential for a predisposition to accelerated adaptation of resistant populations into account.

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TABLE 5. Selection of various *Venturia inaequalis* phenotypes during management of apple scab with dodine and fenarimol under controlled orchard conditions^a

Treatment	n	Frequency of isolates (%) ^b		
		Dod R-Fen S	Dod S-Fen R	Dod R-Fen R
Control ^c	142	8.5	9.9	1.4
Dodine	150	16.0 (0.05)	12.7 (0.32)	9.2 (0.003)
Fenarimol	149	12.1 (0.31)	18.8 (0.04)	7.4 (0.01)

^a Experimental details and results of the experiments described previously (18).

^b Isolate sensitivities determined in 1992, 1993, and 1994 were combined. Phenotype patterns of isolates resistant (R) or sensitive (S) to dodine (Dod) or fenarimol (Fen). Isolates with relative growth >90 or >80 were rated as dodine- or fenarimol-resistant, respectively (20,21). *P* values given in parentheses reflect the comparison of counts of resistant phenotypes with respective counts in the subpopulation derived from nontreated controls (log-linear model).

^c Isolates were sampled from nontreated trees.

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