Baseline Sensitivities of *Venturia inaequalis* to Sterol Demethylation Inhibitors

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ABSTRACT

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A set of monoconidial isolates of *Venturia inaequalis* representing a wild-type population was used to determine the baseline sensitivities and sensitivity distributions for several sterol demethylation inhibitors used in the control of apple scab. Baseline sensitivities expressed as ED_{50} values for growth of mycelia were 0.008 $\mu g\ ml^{-1}$ (flusilazole), 0.03 $\mu g\ ml^{-1}$ (penconazole and pyrifenox), 0.04 $\mu g\ ml^{-1}$ (fenarimol), 0.05 $\mu g\ ml^{-1}$ (bitertanol and tebuconazole), and 0.07 $\mu g\ ml^{-1}$ (myclobutanil). The resistance factors calculated from ED_{50} values determined for a resistant baseline isolate of *V. inaequalis* were variable, ranging from 15 for flusilazole to 3 for pyrifenox.

Sterol demethylation inhibitors (DMIs) constitute a class of fungicides with increasing worldwide importance in the control of plant diseases (8,10,15). Thus far, clearly documented cases of development of DMI resistance have been restricted to powdery mildew of cereals and cucumber (1,6,9). In addition, DMI-resistant phenotypes have been isolated from other pathogen populations (1), including *Venturia inaequalis* (Cooke) G. Wint. (2–4,13, 14,16). The presence of resistant phenotypes in field populations, however, is not necessarily related to practical resistance, since the frequency distribution of DMI-sensitivities is continuous in character and ranges from highly sensitive to resistant (1,6,11,12). For example, the sensitivities detected within a wild-type population of *V. inaequalis* to the DMI flusilazole were separated by a factor of almost 300 (13). This broad sensitivity variation in unexposed populations apparently has no effect on the initial performance of these fungicides. Under the selection force of a DMI, however, the mean sensitivity of a fungal population can shift toward reduced sensitivity, and the proportion of resistant phenotypes may reach a level where satisfactory disease control is no longer provided (1,6,11,12).

Although failures of DMIs in the control of apple scab under commercial orchard conditions has not been a widely recognized problem, poor performance of fenarimol and triflumizole was reported from a commercial orchard site in Austria where DMI fungicides had been intensively used since 1979 (3). Furthermore, unsatisfactory disease control in research orchards with prolonged histories of DMI use has been correlated with reduced sensitivities of isolates of *V. inaequalis* collected from these sites (2,4). In response to these potential signs of eroding performance, a preventive antiresistance strategy for the use of DMIs against *V. inaequalis* has been proposed (9). According to this strategy, DMIs should be restricted to one early-season application, followed by mixtures of DMIs with a protectant fungicide active against *V. inaequalis*.

Because experimental data and past experience in support of this mixture strategy are lacking and alternative strategies might be developed in the future, field monitorings of fungal populations will be required to correlate the levels of disease control achieved under various management conditions with the sensitivity distributions of pathogen populations at particular sites. These monitoring efforts must be based on baseline sensitivities found before the widespread use of DMIs and on monitoring procedures sensitive enough to detect shifts of continuous sensitivity distributions toward decreased mean sensitivities. Both questions were addressed in a previous study on the distribution of flusilazole sensitivities present in wild-type populations of *V. inaequalis* (13). The relative mycelial growth of 50 monoconidial isolates at a discriminatory flusilazole dose slightly higher than the mean sensitivity was proposed as a simplified yet sensitive monitoring procedure. The present study was initiated to determine comparable baseline sensitivities and discriminatory monitoring concentrations for other DMI fungicides used in the control of apple scab.

MATERIALS AND METHODS

Materials and fungal cultures. Sources and chemical purities of flusilazole, fenarimol, myclobutanil, tebuconazole, penconazole, and pyrifenox have been described elsewhere (7). Bitertanol (95% purity) was obtained from Bayer AG, Leverkusen, Germany. Potato-dextrose agar (PDA) and agar were obtained from Difco Laboratories, Detroit. Isolates of *V. inaequalis* were representatives of the baseline isolates collected in New York in 1988 (13). Mycelia of these mono-

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conidial isolates had been stored on PDA slants under mineral oil at 4 C.

Sensitivity tests. The sensitivities to DMIs were determined according to a procedure described elsewhere (13). Two agar plugs (3 mm diameter) cut from mycelial colonies were transferred to plates containing 20 ml of PDA with either no DMI or DMIs at a range of suitable concentrations determined in preliminary experiments. The fungicides were dissolved in acetone before being mixed with PDA cooled to 60 C. In all cases (including unamended control plates), the final acetone concentration was 0.1% (v/v). The mean colony diameter (minus the diameter of the inoculation plug) was determined after incubation for 4 wk at 20 C and expressed as percentage of the mean diameter for the nontreated control. The ED_{50} values were calculated by regressing the relative growth (colony diameter on DMI-amended medium divided by the diameter on unamended medium × 100) against the log of the fungicide concentration.

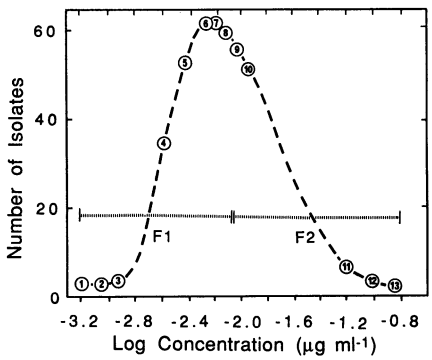

Fig. 1. Position of 13 baseline isolates of *Venturia inaequalis* on a frequency distribution of ED_{50} values determined for 300 wild-type phenotypes (13).

Table 1. Sensitivities of baseline isolates of *Venturia inaequalis* to sterol demethylation inhibitors

	ED_{50} ($\mu g\ ml^{-1}$)		
Isolate[a]	Flusilazole	Fenarimol	Myclobutanil
1	0.0004	0.0045	0.0058
2	0.0007	0.0056	0.013
3	0.0012	0.015	0.019
4	0.0031	0.022	0.034
5	0.0038	0.027	0.052
6	0.0065	0.038	0.045
7	0.0070	0.038	0.058
8	0.0073	0.032	0.054
9	0.0092	0.050	0.075
10	0.012	0.058	0.13
11	0.068	0.11	0.43
12	0.10	0.18	0.54
13	0.12	0.30	0.66
F1[b]	19	9	12
F2[c]	15	7	9

[a] Numbers of isolates correspond to numbers shown in Figure 1.
[b] Baseline ED_{50}/lowest ED_{50}.
[c] Highest ED_{50}/baseline ED_{50}

RESULTS AND DISCUSSION

Baseline sensitivities of *V. inaequalis* to fenarimol and myclobutanil, both DMI fungicides registered for control of apple scab in the United States, were determined with a set of monoconidial isolates derived from an extensive study on the variance of flusilazole sensitivities in wild-type populations (13). The position of selected baseline isolates on the lognormal frequency distribution of sensitivities determined for flusilazole is shown in Figure 1. Emphasis was placed on isolates found at high frequencies and on representatives for highest and lowest sensitivities. Over long-term storage at low temperatures under mineral oil, some but not all of the resistant isolates converted to more sensitive ED_{50} values. This important observation is currently under systematic investigation. The set of isolates used in this study showed stable sensitivities.

Table 1 compares ED_{50} values for flusilazole, myclobutanil, and fenarimol determined for the 13 baseline isolates. The relative ranking of ED_{50} values, ranging from highly sensitive to resistant, generally did not differ for the three DMIs, although slight variations were apparent for some of the isolates representing the highest frequencies found in wild-type populations. The mean baseline sensitivity to flusilazole, calculated as the mean ED_{50} value determined for isolates 5–10, was 0.0076 $\mu g\ ml^{-1}$ and, thus, very close to the mean sensitivity (0.008 $\mu g\ ml^{-1}$) determined for 300 baseline isolates (13). Consequently, this set of isolates was suitable for calculations of comparable baseline sensitivities to myclobutanil and fenarimol (Table 2). A reduced set of isolates (7 and 9) was used to determine the corresponding baseline sensitivities for penconazole, bitertanol, pyrifenox, and tebuconazole, all DMIs in use or being developed for the control of apple scab.

The ranking of intrinsic activities as indicated by the mean baseline sensitivity was flusilazole > penconazole = pyrifenox > fenarimol > bitertanol = tebuconazole > myclobutanil (Table 2). All compounds, however, were highly active, with baseline ED_{50} values < 0.1 $\mu g\ ml^{-1}$. A monitoring procedure based on the mean relative growth at a single flusilazole concentration of 0.01 $\mu g\ ml^{-1}$ has been proposed recently (13). Corresponding discriminatory monitoring doses slightly higher than the mean baseline sensitivities are suggested in Table 2 for the DMIs tested in this study. Results from our own preliminary field studies have validated the suitability of the concentrations given for flusilazole, myclobutanil, and fenarimol (*unpublished*).

The ED_{50} values for isolates of *V. inaequalis* considered sensitive to DMIs have been reported previously. Table 3 compares these published data with baseline sensitivities determined in this study. The sensitivities reported by Fiaccadori et al (2) for fenarimol, bitertanol, and penconazole were based on the mean ED_{50} values of 12 monoconidial isolates derived from unexposed

Table 2. Baseline data of sterol demethylation inhibitors (DMIs) for *Venturia inaequalis*

DMI	Baseline sensitivity	Monitoring dose[a]	ED_{50} (resistant)[b]	F2[c]
Flusilazole	0.008[d]	0.01	0.12	15
Penconazole	0.03[e]	0.05	0.15	5
Pyrifenox	0.03[e]	0.05	0.10	3
Fenarimol	0.04[d]	0.05	0.30	8
Bitertanol	0.05[e]	0.08	0.20	4
Tebuconazole	0.05[e]	0.08	0.35	7
Myclobutanil	0.07[d]	0.1	0.66	9

[a] Proposed discriminatory dose ($\mu g\ ml^{-1}$) for monitorings based on relative growth (13).
[b] ED_{50} value ($\mu g\ ml^{-1}$) of isolate 13.
[c] Resistant ED_{50}/baseline ED_{50}.
[d] Mean ED_{50} value ($\mu g\ ml^{-1}$) of isolates 5–10.
[e] Mean ED_{50} value ($\mu g\ ml^{-1}$) of isolates 7 and 9.

Table 3. Comparison of reported baseline sensitivities of *Venturiaa inaequalis* to sterol demethylation inhibitors (DMIs)

	Sensitivities[a]			
DMI	Table 2	Fiaccadori et al (2)	Thind et al (16)	Hermann et al (3)
Flusilazole	0.008	...	0.09	...
Penconazole	0.03	0.02	0.02	0.2
Pyrifenox	0.03	...	...	0.2
Fenarimol	0.04	0.04	0.03	0.4
Bitertanol	0.05	0.05	0.4	...
Tebuconazole	0.05	...	...	...
Myclobutanil	0.07	...	...	...

[a] Expressed as ED_{50} values ($\mu g\ ml^{-1}$).

orchards in the Netherlands and Italy. They are in excellent agreement with the sensitivities determined by us, indicating that sensitivity distributions to DMIs in New York and Europe are very similar. The sensitivity data are less comparable with ED_{50} values reported by others. Based on three isolates classified as DMI-sensitive, Thind et al (16) reported ED_{50} values for fenarimol and penconazole very similar to those found in our study. However, the same isolates were 10 times less sensitive to flusilazole and bitertanol. A similar drastic departure from relative sensitivity rankings has not been observed with our set of baseline isolates. The ED_{50} values for penconazole, pyrifenox, and fenarimol reported by Hermann et al (3) for a single isolate of *V. inaequalis* collected before the introduction of DMIs was approximately 10 times higher than those found by others.

Although the apparent discrepancies among independent studies deserve critical evaluation, they exemplify the importance of baseline data derived from isolates clearly identified as representatives of sensitivities found at highest frequencies in unexposed wild-type populations. Reliable baseline sensitivities in combination with population sensitivities at sites where performance of DMIs has declined would indicate the extent of tolerable shifts and thus are crucial for predictive monitoring programs. The extent of population shifts beyond levels of unsatisfactory disease control has not yet been established. The set of baseline data presented in this study will provide the basis for studies on this open question.

Because DMIs are cross-resistant to each other, monitorings based on only one will be indicative for the whole group. As discussed by Smith et al (13), the sample size requirements depend on the variance of sensitivities and might not be identical for all DMIs. Indeed, the variance for the DMIs tested in this study differed. Sensitivity distributions were symmetrical, as expressed by the similarities of factors F1 and F2 (Table 1). The extent of sensitivity variations (F1 × F2), however, ranged from a factor of 300 for flusilazole to 110 for myclobutanil and 60 for fenarimol (Table 1). When F2 values were compared, a similar variability was found for penconazole, pyrifenox, bitertanol, and tebuconazole (Table 2). This value is closely related to the resistance factor, which has been shown to be variable for other pathogens and various DMIs (5,7). For *V. inaequalis*, F2 ranged from 15 for flusilazole to 3 for pyrifenox (Table 2). We should emphasize, however, that phenotypes with higher levels of resistance appear to exist but are too infrequent to be identified within a limited sample of isolates collected from baseline populations. For example, Thind et al (16) reported ED_{50} values determined for a DMI-resistant isolate of *V. inaequalis* that were considerably higher than those reported for the resistant baseline isolate used in this study. Regardless of the absolute value of resistance factors, the variance of sensitivities found in wild-type populations is different for the DMIs tested. Because the sample size required in monitorings decreases with a decreasing variance (13), and because the variance was highest for flusilazole, a sample size of 50 monoconidial isolates proposed for monitorings based on flusilazole (13) will be sufficient for all DMIs listed in Table 2.

The impact of variable resistance factors on the performance of particular DMIs under field conditions is hard to predict. The highest intrinsic activity determined for flusilazole might be partly compensated for by a wider range of sensitivities. For example, flusilazole was four times more active than pyrifenox, based on the baseline sensitivity. Both DMIs, however, exerted equal activity on a resistant phenotype (Table 2). Because application rates must be adjusted to the less sensitive part of the sensitivity distribution, both compounds are more similar than the different baseline activities suggest. However, a wider sensitivity distribution could be advantageous under conditions of increasing frequencies of DMI-resistant phenotypes. The proportion of resistant phenotypes shifting at a given time of selection pressure beyond a threshold level of satisfactory disease control might be higher for a DMI with a narrow sensitivity distribution. Most of these phenotypes would still be sensitive to a DMI with a broader range of sensitivities, and the development of practical resistance could be less abrupt and severe than for a DMI with a narrow range of sensitivity distributions. Although clear cross-resistance among the DMIs active against *V. inaequalis* exists, the impact of variable resistance factors and, thus, sensitivity ranges on disease control remains to be investigated.

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