Reduced Sensitivity to Sterol-Inhibiting Fungicides in Field Isolates of *Venturia inaequalis*

V. F. Stanis and A. L. Jones

Department of Botany and Plant Pathology and the Pesticide Research Center, Michigan State University, East Lansing 48824.
Michigan Agricultural Experiment Station Journal Article 11598
Accepted for publication 6 May 1985 (submitted for electronic processing).

ABSTRACT

Stanis, V. F., and Jones, A. L. 1985. Reduced sensitivity to sterol-inhibiting fungicides in field isolates of *Venturia inaequalis*. Phytopathology 75:1098-1101.

For eight sterol-inhibiting fungicides, minimal inhibitory concentrations for preventing colony formation by individual conidia were four to eight times higher for isolates of *Venturia inaequalis* from one West German orchard than for isolates from either a second West German orchard or orchards in the United States. Reduced sensitivity was exhibited to BAS 454 06 F (1-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl)), bitertanol, CGA 71818 (1-[2-(2,4-dichlorophenyl)-pentyl]-1*H*-1,2,4-triazole), DPX H6573 (*bis*(4-fluorophenyl)methyl(1*H*-1,2,4-triazol-1-yl methyl)silane), etaconazole, fenarimol, Ro 15-1297 (1-(2,4-dichlorophenyl)-2-(3-pyridinyl)-ethanone *O*-methyloxime), and triflumizole. Isolates with reduced sensitivity to sterol inhibitors did not show increased sensitivity to dodine. Genetic analysis of nine isolates with reduced sensitivity indicated that reduced sensitivity was determined by a single gene.

Additional key words: apple scab

Several sterol-inhibiting fungicides are now available for agricultural use, and a number of others are being developed. These fungicides are a chemically diverse group of compounds that specifically inhibit ergosterol biosynthesis (12,16). Most of the sterol-inhibiting fungicides being developed on apple are effective at very low concentrations against apple scab, powdery mildew, and cedar-apple rust, diseases that must be controlled in many apple production areas during the first 2–3 mo of the growing season (21). In addition to this useful spectrum of activity, these compounds have exhibited excellent postinfection control activity (14,18). They are being proposed as alternatives to dodine and to benomyl where dodine-resistant and/or benomyl-resistant strains of *Venturia inaequalis* (Cke.) Wint. have developed. Because of these favorable attributes, sterol-inhibiting fungicides may be used extensively on apple in the near future.

The majority of the sterol inhibitors being developed for use on apple are inhibitors of C-14 demethylation in sterol biosynthesis (12,16). Under laboratory conditions, mutants resistant to inhibitors of C-14 demethylation are readily induced and cross-resistance often occurs (1,4,5). Naturally occurring strains with

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "*advertisement*" in accordance with 18 U.S.C. § 1734 solely to indicate this fact.

reduced sensitivity to this group of sterol-inhibiting fungicides are less common, but they have been reported in populations of *Erysiphe graminis* f. sp. *hordei* and of *Sphaerotheca fuliginea* collected from the greenhouse and field (7,13,20). As yet, there is no report of reduced sensitivity to sterol-inhibiting fungicides in naturally occurring strains of *V. inaequalis*.

Recently, from the Federal Republic of Germany we obtained field isolates of *V. inaequalis* that formed sporulating colonies on media amended with sterol-inhibiting fungicides, while isolates from other sources failed to grow at similar fungicide concentrations. This study was conducted to determine if the differential response of these isolates was stable in culture, extended across a range of sterol-inhibiting fungicides, and whether this trait was genetically controlled. We also tested the response of these isolates to dodine because fenarimol resistance in the nonpathogen *Aspergillus nidulans* was reported to be negatively correlated with dodine resistance (5).

MATERIALS AND METHODS

Cultures. Twelve monoconidial isolates of *V. inaequalis* were from two apple orchards in the Federal Republic of Germany. Nine of the isolates, designated B1 to B9, were collected from one orchard, and three isolates, designated W10 to W12, were from a second orchard. Each isolate was from a different leaf lesion. Sterol-inhibiting fungicides had been used experimentally in both orchards. The following monoconidial isolates were from Michigan: WB, WRR, GAVIN 29, and GAVIN 32. Isolates WB and WRR were opposite mating types and GAVIN 29 and GAVIN 32 were dodine resistant. The Michigan isolates were collected from orchards where no sterol-inhibiting fungicides had been used.

Isolates were grown at 18 C on modified potato-dextrose agar (modified PDA: 40 g of potatoes steamed for 45 min in 200 ml of distilled water and then homogenized, 17 g of agar, 5 g of dextrose, and distilled water to make a final volume of 1 L)(2). At 4- to 6-wk intervals, single conidia were subcultured.

Fungicides and chemicals. The fungicides used in this study were: 1-(2,4-dichlorophenyl)-2-(1*H*-1,2,4-triazol-1-yl) (BAS 454 06 F 25% EC) from BASF Wyandotte Corp., Parsippany, NJ; bitertanol (Baycor 25% WP) from Bayer AG, Leverkusen, Federal Republic of Germany; 1-[2-(2,4-dichlorophenyl)-pentyl]-1*H*-1,2,4-triazole (CGA 71818 10% WP), and etaconazole (Vangard 10% WP) from Ciba-Geigy Corp., Greensboro, NC; dodine (100% technical grade) from American Cyanamid Co., Princeton, NJ; *bis*(4-fluorophenyl)methyl(1*H*-1,2,4-triazol-1-yl methyl)silane (DPX H6573 40% EC) from E.I. duPont de Nemours and Co., Inc., Wilmington DE; fenarimol (Rubigan 12.5% EC) from Eli Lilly and Co., Greenfield, IN; 1-(2,4-dichlorophenyl)-2-(3-pyridinyl)-ethanone *O*-methyloxime (Ro 15-1297 48% EC) from Maag Agrochemicals, Vero Beach, FL; and triflumizole (A-815 30% WP) from Uniroyal, Naugatuck, CT.

Minimal inhibitory concentrations. Minimal fungicide concentrations needed to inhibit colony formation were determined by plating conidia on 2% water agar and after 12–24 hr transferring individual germinated conidia to 100 × 15-mm petri dishes containing PDA (Difco Laboratories Inc., Detroit, MI) amended with a series of fungicide concentrations. Concentrations ranged from 0 to 5.0 μg/ml for all fungicides. With 95% ethanol added to assist in dissolution, fungicides were added to molten PDA (42 C) after sterilization. The final ethanol concentration never exceeded 1% in either treatment or control. Up to 12 isolates were tested simultaneously in each petri dish (Fig. 1). Colony growth was evaluated after 3 wk of incubation at 20–22 C.

Sensitivity to dodine. Response to dodine was tested using two methods. With the first method, conidia were germinated on 2% water agar and transferred individually to dodine-amended PDA. Dodine concentrations ranging from 0 to 1.0 μg/ml in 0.1 μg/ml increments were prepared by adding dodine suspended in ethanol to molten PDA (42 C) after sterilization. The concentration of ethanol never exceeded 1% in either treatment or control. Dodine resistant isolates GAVIN 29 and GAVIN 32 were included for comparison. Colony growth was evaluated after 3 wk.

With the second method, isolates were compared for inhibition of growth around assay disks treated with 0, 50, and 300 μg of dodine per milliliter. Sterile 13-mm-diameter assay disks (Schleicher and Schuell, Inc., Keene, NH) were saturated with 100 μl of distilled water or dodine solution and allowed to dry. For each isolate tested, a 2- to 3-wk-old colony was excised from its agar substrate and homogenized in 4 ml of sterile distilled water in a 7-ml tissue grinder. Aliquots (0.5 ml) of homogenate were used to seed the surface of PDA in each of three 100 × 15-mm petri dishes, and a treated disk was placed in the center of each dish and pressed lightly to ensure contact with the agar. Diameters of zones of inhibition were measured after 25 days of incubation at 20–22 C.

Crosses. Isolates were crossed using the procedure of Keitt and Langford (9), except colony homogenates rather than spore suspensions were used as inoculum. Individual 2- to 3-wk-old colonies were removed from their agar substrates and homogenized in 2 ml of sterile distilled water in a 7-ml tissue grinder. A 0.5-ml aliquot of homogenate of each member of the cross was added to a 100 × 15-mm petri dish, 30 ml of warm (42 C) modified PDA with apple leaf decoction (9) was added, and the homogenate and agar were mixed by swirling the plate. The plates were incubated 7–10 days at 18 C until mycelial growth was evident on the surface of the agar, then sealed with Parafilm (American Can Co., Greenwich, CT), inverted, and held at 8 C for 5–6 mo as required for pseudothecia formation and ascospore maturation. All isolates used in crosses were also selfed to confirm self-incompatibility.

Testing of progenies. For each fertile pairing, 12 to 20 pseudothecia were removed from the agar substrate with the aid of

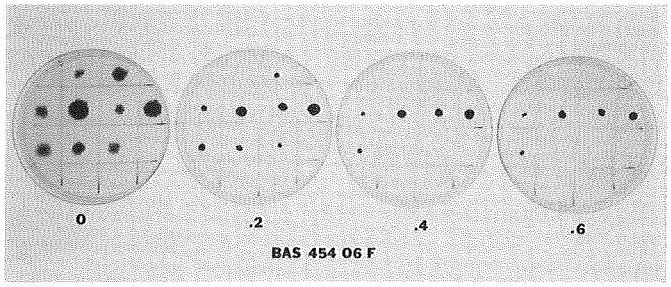

Fig. 1. Response of *Venturia inaequalis* on potato-dextrose agar amended with 0, 0.2, 0.4, or 0.6 μg of BAS 454 06 F per milliliter. Five isolates with reduced sensitivity to BAS 454 06 F and four sensitive isolates were tested simultaneously at each concentration. Three weeks after inoculation with single germinated conidia, isolates with reduced sensitivity showed growth at the two higher concentrations, but sensitive isolates did not.

TABLE 1. Minimal fungicide concentrations for preventing colony formation by germinated conidia of *Venturia inaequalis* with two levels of sensitivity to sterol-inhibiting fungicides

Fungicide	Minimal inhibitory concentration (μg/ml)[a]		Suggested discriminatory concentration[b] (μg/ml)
	Sensitive isolates	Isolates with reduced sensitivity	
BAS 454 06 F	0.4	2.0	0.9-1.0
Bitertanol	1.0	5.0	0.5-1.0
CGA 71818	0.2	0.8	0.3-0.4
Etaconazole	0.1	0.6	0.3-0.4
DPX H6573	0.1	0.6	0.2-0.3
Fenarimol	0.4	3.0	1.0-1.5
Ro 15-1297	0.1	0.4	0.2
Triflumizole	0.2	1.0	0.4-0.5

[a] Data are means of three replicated tests with field isolates that exhibited reduced sensitivity to these fungicides and with sensitive field isolates. Similar results were also obtained in numerous tests with single-ascospore isolates derived from crosses between sensitive and less-sensitive strains.
[b] Suggested discriminatory concentrations inhibit colony formation by conidia from sensitive isolates but not that by conidia from isolates with reduced sensitivity to sterol-inhibiting fungicides.

a needle and a dissecting microscope and crushed in 1 ml of sterile distilled water. Portions of the resultant ascospore suspension were plated on 2% water agar and incubated at 20–22 C for 12–24 hr. Randomly chosen germinated ascospores were transferred to individual culture tubes containing modified PDA. After 2–3 wk of growth, bits of mycelium (1 mm in diameter) were taken from the mid-radius area of the colonies and inoculated onto PDA and PDA amended with 1.5 μg of fenarimol per milliliter. Growth was evaluated after 3 wk of incubation at 20–22 C. Selected single-ascospore colonies were also tested for sensitivity to other sterol-inhibiting fungicides and for sensitivity to dodine.

RESULTS

Minimal inhibitory concentrations. Although colony size of all isolates tested diminished with increasing fungicide concentration, West German isolates B1 to B9 grew at higher concentrations of each sterol inhibitor than did West German isolates W10 to W12 or United States (Michigan) isolates WB, WRR, GAVIN 29, and GAVIN 32. Single-ascospore isolates derived from crosses between sensitive and less-sensitive isolates behaved either like their sensitive or less-sensitive parent. Within the less-sensitive and sensitive groups, the response of individual members to different concentrations of each sterol inhibitor was uniform (Fig. 1, Table 1).

Minimal inhibitory concentrations for differentiating isolates with reduced sensitivity were selected for each sterol inhibitor (Table 1). At these concentrations, sensitive isolates failed to grow, while less-sensitive isolates formed colonies. Except on media amended with Ro 15-1297, sporulation of colonies was observed in 3–4 wk.

Response to dodine. When germinated conidia were transferred to dodine-amended PDA, growth of isolates with reduced sensitivity to sterol inhibitors did not differ from the growth of sensitive isolates. Both groups showed the same diminution in colony size with increasing dodine concentration and cessation of growth at 0.2–0.3 μg of dodine per milliliter. The dodine-resistant isolates, GAVIN 29 and GAVIN 32, grew at 0.5 and 0.7 μg of dodine per milliliter but not at 1.0 μg/ml.

In tests with assay disks, no zones of inhibition developed around disks treated with water as a control. Growth was inhibited around disks treated with solutions containing dodine. At both 50 and 300 μg of dodine per milliliter, zones of inhibition were slightly less for the isolates with reduced sensitivity to the sterol inhibitors than for sensitive isolates (Table 2). However, according to Student's *t*-test, differences in mean diameters of zones of inhibition for the two groups were not significantly different at $P = 0.05$.

Analysis of crosses. None of the isolates produced pseudothecia when selfed, thereby confirming their self-incompatibility. Single-ascospore isolates derived from fertile pairings were readily separated into two groups based on growth on PDA containing 1.5 μg of fenarimol per milliliter. At this concentration, sensitive isolates failed to grow, while less-sensitive isolates formed colonies. When crosses were made between sensitive isolates, isolates W10, W11, and W12 were fertile with WRR, indicating that WB, W10, W11, and W12 were of the same mating-type (Table 3). All 361 ascospores from the three crosses among four sensitive isolates were sensitive.

To determine whether reduced sensitivity was controlled by more than one gene, 1,867 ascospores were tested from nine crosses between sensitive and less-sensitive isolates (Table 3). Isolates B1, B2, and B5 were compatible with WB, and isolates B3, B4, B6, B7, B8, and B9 were compatible with WRR. In all but one cross, sensitive progeny were found in a 1:1 ratio with less-sensitive progeny. The only ratio deviating from the 1:1 ratio was that of cross B5 × WB.

To determine whether the mutations for reduced sensitivity to sterol-inhibiting fungicides occurred at the same locus, crosses between less-sensitive isolates were examined (Table 3). A total of 1,394 ascospores were tested from six compatible pairings resulting from all possible pairings of isolates B1 to B5. No sensitive progeny were detected. From the analysis of crosses between sensitive and less-sensitive isolates, and between the less-sensitive isolates, it was concluded that, in the isolates studied, reduced sensitivity to the sterol-inhibition fungicides was determined by a single gene. The gene for reduced sensitivity was not linked to mating type.

TABLE 2. Inhibition of mycelial growth surrounding assay disks treated with two concentrations of dodine and placed on agar seeded with sensitive isolates of *Venturia inaequalis* or with isolates that exhibited reduced sensitivity to sterol-inhibiting fungicides

	Inhibition zone (mm) caused by dodine at:			
	50 μg/ml		300 μg/ml	
Cross	Sensitive isolates	Less-sensitive isolates	Sensitive isolates	Less-sensitive isolates
B4 × WRR	21.0 ±4.6[a]	17.4 ± 3.1	31.8 ±4.4	28.6 ±3.8
B5 × WB	18.2 ±1.8	15.0 ±1.0	32.0 ±1.0	23.6 ±3.3
B6 × WRR	18.6 ±2.5	15.0 ±1.9	29.2 ±3.8	24.2 ±4.8
B7 × WRR	14.4 ±1.7	14.4 ±1.5	23.2 ±5.8	23.0 ±2.0
B8 × WRR	17.4 ±4.0	15.2 ±3.0	27.2 ±4.5	25.4 ±2.9
B9 × WRR	22.0 ±2.9	18.0 ±3.3	36.8 ±7.3	27.8 ±3.8

[a] Mean diameters and standard deviations are given for zones of inhibition of five sensitive isolates and five less-sensitive isolates from each cross. Values include 13-mm-diameter of the assay disk.

TABLE 3. Segregation of single-ascospore isolates of *Venturia inaequalis* into less-sensitive and sensitive phenotypes according to growth on potato-dextrose agar amended with fenarimol at 1.5 μg/ml

	Progeny tested (no.)			
		Penotype		
Cross	Total	Less-sensitive	Sensitive	χ^2 values (1:1)[a]
Sensitive × sensitive				
W10 × WRR	90	0	90	
W11 × WRR	99	0	99	
W12 × WRR	172	0	172	
Totals	361	0	361	
Less-sensitive × sensitive				
B1 × WB	252	142	110	4.06
B2 × WB	228	97	131	5.07
B3 × WRR	96	43	53	1.04
B4 × WRR	250	116	134	1.30
B5 × WB	96	68	28	16.70
B6 × WRR	230	97	133	5.63
B7 × WRR	224	124	100	2.57
B8 × WRR	244	103	141	5.92
B9 × WRR	247	136	111	2.53
Totals	1,867	918	949	0.12
Less-sensitive × less-sensitive				
B1 × B3	229	229	0	
B1 × B4	246	246	0	
B2 × B3	240	240	0	
B2 × B4	246	246	0	
B3 × B5	248	248	0	
B4 × B5	185	185	0	
Totals	1,394	1,394	0	

[a] The expected chi-square value, $P = 0.01$, is 6.63.

DISCUSSION

The minimal concentration of sterol-inhibiting fungicides necessary to inhibit the growth of the *V. inaequalis* isolates B1 to B9 from West Germany was four to eight times the concentration necessary to inhibit the growth of isolates of *V. inaequalis* from a second West German orchard and from orchards in the United States. This differential response in sensitivity was not lost or changed in culture and was genetically controlled. The level of variation between less-sensitive and sensitive isolates was comparatively small and in the order of variation reported for dodine-resistant and sensitive *V. inaequalis* (11,22). There was no

evidence for high levels of resistance of the type reported for strains of benomyl-resistant *V. inaequalis* (8,10).

Because reduced sensitivity was controlled by a single gene, the potential for a buildup of these strains in the presence of prolonged exposure to inhibitors of C-14 demethylation is greater than if several genes were required (3,6). As isolates with reduced sensitivity came from the same source and responded uniformly in our tests, the same gene probably controlled decreased sensitivity in all the isolates. If additional genes for reduced sensitivity exist, higher levels of resistance could result. Such a system would be similar to that already described for dodine resistance in *V. inaequalis* (11,22).

Although it is still too early to make uneqivocal statements about the fitness of the less-sensitive strain, there was no evidence to suggest that it lacked fitness. It was isolated from infected apple leaves and thus was pathogenic at the time of isolation. The isolates grew and sporulated well in culture, which suggests they may have sufficient fitness for survival among populations with sensitive strains. No problem such as abortion factors were encountered in making sexual crosses between the strains (23). Since *V. inaequalis* completes the sexual process annually, there is enormous potential for acquiring greater fitness. Unless decreased sensitivity is closely linked with reduced fitness, previous experience with dodine-resistant and benomyl-resistant strains of *V. inaequalis* indicates resistant forms are often fit to survive by the time they can be detected.

De Waard and van Nistelrooy (4,5) reported that two laboratory-induced, fenarimol-resistant mutants of *A. nidulans* were slightly more sensitive to dodine than a wild-type fenarimol-sensitive strain of the fungus. They postulated that dodine-resistant strains of *V. inaequalis* may be more sensitive to sterol-inhibiting fungicides than sensitive strains, and that field isolates with reduced sensitivity to the sterol inhibitors may be more sensitive to dodine (5). In our studies, strains of *V. inaequalis* with reduced sensitivity to fenarimol were equally as sensitive to dodine as sensitive strains, and no correlation between reduced sensitivity to sterol inhibitors and increased dodine sensitivity was observed. Also, dodine-resistant *V. inaequalis* were not more sensitive to sterol inhibitors. This suggests that with *V. inaequalis*, dodine may not provide any particular benefit in combating population shifts toward reduced sensitivity to sterol inhibitors.

None of the sterol-inhibiting fungicides evaluated in this study are presently in commercial use on apple in the United States and only a few are in commercial use in other areas of the world. In regions where dodine-resistant and benomyl-resistant strains exist, the rapid incorporation of the sterol-inhibiting fungicides into present programs is needed to control scab when disease pressure is high or in emergency situations. In these regions, the sterol-inhibiting fungicides are the only fungicides remaining with excellent curative action against apple scab. The present work indicates the suggested discrimination concentration given in Table 1 could be used to monitor populations of *V. inaequalis* for shifts in sensitivity. We suggest that fenarimol be used for routine monitoring because slight variations in fungicide concentration from test to test are less critical. Monitoring for less-sensitive strains of *V. inaequalis* as these fungicides are introduced would provide an early assessment of the potential for resistance risk. Also, as noted by Staub and Sozzi (17), monitoring can be used to quell rumors of resistance when poor disease control is not the fault of the chemical and to make the right decision when resistance is the cause. Monitoring can also be used to validate the reliability of any antiresistance strategies that are adapted.

The detection of a less-sensitive strain of *V. inaequalis* in an experimental orchard is a warning of potential problems in practice from the intensive use of the C-14 demethylation group of sterol-inhibiting fungicides. Other indications of potential problems included: the decreased sensitivity to biteranol, fenarimol, and triforine of *S. fuliginea* in the Netherlands and Israel (13); the decreasing sensitivity to triazole fungicides of field populations of *E. graminis* f. sp. *hordei* in the United Kingdom (19,20); and the apparent appearance of triadimenol-insensitive strain of *Pyrenophora teres* on barley in New Zealand (15). Because the C-14 demethylation group of sterol-inhibiting fungicides is at risk, cooperation among all manufactureres is required to develop and implement a strategy for avoiding resistance in *V. inaequalis*.

LITERATURE CITED

1. Barug, D., and Kerkenaar, A. 1979. Cross-resistance of UV-induced mutants of *Ustilago maydis* to various fungicides which interfere with ergosterol biosynthesis. Meded. Fac. Landbouwwet. Rijksuniv. Gent. 44:421-427.
2. Boone, D. M., and Keitt, G. W. 1956. *Venturia inaequalis* (Cke.) Wint. VIII. Inheritance of color mutant characters. Am. J. Bot. 43:226-233.
3. Dekker, J. 1976. Acquired resistance to fungicides. Annu. Rev. Phytopathol. 14:405-428.
4. de Waard, M. A., and Gieskes, S. A. 1977. Characterization of fenarimol-resistant mutants of *Aspergillus nidulans*. Neth. J. Plant Pathol. 83 (Suppl. 1):177-188.
5. de Waard, M. A., and van Nistelrooy, J. G. M. 1983. Negatively correlated cross-resistance to dodine in fenarimol-resistant isolates of various fungi. Neth. J. Plant Pathol. 89:67-73.
6. Gilpatrick, J. D. 1983. Management of resistance in plant pathogens. Pages 735-767 in: Pest Resistance to Pesticides. G. P. Georghiou and T. Saito, eds. Plenum Press, New York. 809 pp.
7. Huggenberger, F., Collins, M. A., and Skylakakis, G. 1984. Decreased sensitivity of *Sphaerotheca fuliginea* to fenarimol and other ergosterol-biosynthesis inhibitors. Crop Prot. 3:137-149.
8. Katan, T., Shabi, E., and Gilpatrick, J. D. 1983. Genetics of resistance to benomyl in *Venturia inaequalis* from Israel and New York. Phytopathology 73:600-603.
9. Keitt, G. W., and Langford, M. H. 1941. *Venturia inaequalis* (Cke.) Wint. I. A groundwork for genetic studies. Am. J. Bot. 28:805-820.
10. Kiebacher, J., and Hoffmann, G. M. 1980. Qualitative und quantitative Untersuchungen zur Resistenz von *Venturia inaequalis* gegen Benzimidazol-Fungizide. Z. Pflanzenkrankh. Pflanzenschutz 87:705-716.
11. Polach, F. J. 1973. Genetic control of dodine tolerance in *Venturia inaequalis*. Phytopathology 63:1189-1190.
12. Ragsdale, N. N. 1977. Inhibitors of lipid synthesis. Pages 333-363 in: Antifungal Compounds, Vol. II. M. R. Siegel and H. D. Sisler, eds. Marcel Dekker, New York. 674 pp.
13. Schepers, H. T. A. M. 1983. Decreased sensitivity of *Sphaerotheca fuliginea* to fungicides which inhibit ergosterol biosynthesis. Neth. J. Plant Pathol. 89:185-187.
14. Schwabe, W. F. S., Jones, A. L., and Jonker, J. P. 1984. Greenhouse evaluation of the curative and protective action of sterol-inhibiting fungicides against apple scab. Phytopathology 74:249-252.
15. Sheridan, J. E., and Grbavac, N. 1985. Cultivar differences in response to triadimenol seed treatment for control of barley net blotch caused by *Pyrenophora teres*. Plant Dis. 69:77-80.
16. Siegel, M. R. 1981. Sterol-inhibiting fungicides: effects on sterol biosynthesis and sites of action. Plant Dis. 65:986-989.
17. Staub, T., and Sozzi, D. 1984. Fungicide resistance: A continuing challenge. Plant Dis. 68:1026-1031.
18. Szkolnik, M. 1981. Physical modes of action of sterol-inhibiting fungicides against apple diseases. Plant Dis. 65:981-985.
19. Walmsey-Woodward, D. J., Laws, F. A., and Whittington, W. J. 1979. Studies on the tolerance of *Erysiphe graminis* f. sp. *hordei* to systemic fungicides. Ann. Plant Biol. 92:199-209.
20. Wolfe, M. S., Minchin, P. N., and Slater, S. E. 1984. Dynamics of triazole sensitivity in barley mildew, nationally and locally. Pages 465-478 in: Proc. Br. Crop. Conf., 19–22 November 1984, Brighton, England.
21. Yoder, K. S., and Hickey, K. D. 1981. Sterol-inhibiting fungicides for control of certain diseases of apple in the Cumberland-Shenandoah region. Plant Dis. 65:998-1001.
22. Yoder, K. S., and Klos, E. J. 1976. Tolerance to dodine in *Venturia inaequalis*. Phytopathology 66:918-923.
23. Yoder, K. S., Klos, E. J., Nowacka, H., and Bielenin, A. 1982. Inheritance of an ascospore abortion factor in *Venturia inaequalis*. Can. J. Bot. 60:2105-2111.