Fungicide Sensitivity of *Sphaerotheca fuliginea* Populations in the United States

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ABSTRACT

McGrath, M. T., Staniszewska, H., Shishkoff, N., and Casella, G. 1996. Fungicide sensitivity of *Sphaerotheca fuliginea* populations in the United States. Plant Dis. 80:697-703.

Isolates of the cucurbit powdery mildew fungus, *Sphaerotheca fuliginea*, resistant to triadimefon (able to grow on leaf disks treated with 50 μg/ml) and isolates resistant to benomyl (200 μg/ml) were found in commercial production fields and research plots that had not been treated with these fungicides in California, Florida, Georgia, Michigan, North Carolina, and New York in 1993 and 1994. Triadimefon-resistant isolates also were found in nontreated fields in Arizona and Virginia and in treated fields in Texas. Overall, triadimefon-resistant isolates were found in 18 of 19 nontreated fields; benomyl-resistant isolates were found in eight of these fields. The proportion of isolates obtained from nontreated commercial fields that were resistant ranged from 2 to 95% for triadimefon and from 3 to 25% for benomyl. Triadimefon was used more than benomyl for managing cucurbit powdery mildew in the United States in the 1990s. Triadimefon-resistant isolates were less sensitive to two other triazole fungicides not registered for use on cucurbits in the United States when this study was conducted: 91 and 94% of the isolates that were insensitive to propiconazole at 5 μg/ml and myclobutanil at 20 μg/ml, respectively, were resistant to triadimefon. The highest concentration of myclobutanil tolerated by 403 triadimefon-sensitive isolates was 0 to 0.01, 0.1 to 1.0, 2, and 20 μg/ml for 14.5, 49, 35, and 1.5%, respectively. The highest concentration of propiconazole tolerated by 256 triadimefon-sensitive isolates was 0 to 0.01, 0.05, 0.5, and 5 μg/ml for 7, 44, 46, and 3%, respectively. Spearman rank correlation coefficients were 0.7 for these fungicides.

Additional keywords: benzimidazole, fungicide resistance, DMI, SBI, EBI

Management of powdery mildew, caused by *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci, is required to avoid a reduction in yield or market quality for most cucurbit crops (30). Powdery mildew is an important cucurbit disease that occurs every year throughout most production areas of the United States. Fungicides are the only commercially available control option for pumpkin (*Cucurbita pepo* L.), summer squash (*C. pepo* var. *melopepo* (L.) Alef.), and winter squash (*C. pepo* and *C. moschata* (Duchesne) Duchesne ex Poir.). Plant breeders are developing powdery mildew–resistant cultivars; however, it is not anticipated that genetic control will eliminate the need for fungicides. For some crops, an integrated management program may be more effective than genetic control alone. An integrated program also may be an effective strategy to manage development of new races and development of fungicide-resistant isolates. In addition, horticultural characteristics are more important than disease susceptibility for some crops due to consumer demand for variety and novelty in melons (*Cucumis melo* L.) and pumpkins.

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Accepted for publication 11 March 1996.

Publication no. D-1996-0417-06R

Successful management of powdery mildew depends on systemic fungicides to suppress disease development on abaxial leaf surfaces (30). In previous studies, we detected resistance to two systemic fungicides, triadimefon and benomyl, in *S. fuliginea* populations in pumpkin fields where powdery mildew had not been controlled adequately with multiple applications of triadimefon in New York (29) and in Michigan (31). Benomyl-resistant isolates had been detected in New York previously (44). We found triadimefon usage to be associated with a substantial increase over a 2- to 4-week period in the proportion of isolates able to tolerate triadimefon at 50 μg/ml (29). This change in the fungal population was associated with an apparent reduction in fungicide efficacy near the end of the growing season (27,30). Triazole fungicides, such as triadimefon, myclobutanil, and propiconazole, are in the demethylation inhibitor (DMI) group of fungicides, which is one of two subgroups in the ergosterol biosynthesis inhibitor (EBI or SBI) group of fungicides. Benomyl is a benzimidazole fungicide.

The objectives of this study were (i) to determine the sensitivities of *S. fuliginea* populations to benomyl and to triadimefon, especially before fungicide treatment; (ii) to determine the baseline sensitivity of *S. fuliginea* populations in the United States to myclobutanil and to propiconazole, two systemic fungicides that were not registered for use on cucurbits in the United States when this study was conducted; and (iii) to determine whether sensitivity to myclobutanil or to propiconazole is correlated with sensitivities to triadimefon or benomyl. At the time of this study, triadimefon was the predominant systemic fungicide used commercially in the United States for powdery mildew on cucurbits. Information is needed on the baseline sensitivity of *S. fuliginea* to myclobutanil and to propiconazole for two reasons: to assess the potential utility of these fungicides for managing cucurbit powdery mildew and to use in the future (after these fungicides have been registered for several years) as a benchmark of the pathogen population before these fungicides were used commercially. A preliminary report was published (33).

MATERIALS AND METHODS

Powdery mildew populations. The primary goal was to examine populations that had not been exposed to triazole or benzimidazole fungicides and that were in commercial fields from major cucurbit production areas in the United States in order to obtain information on baseline sensitivity. However, most commercial growers apply triadimefon beginning early in disease development, often before symptoms are evident. Consequently, many of the populations examined were from fungicide-treated fields (Tables 1 and 2). Isolates from nontreated research plots were included in the study, although these populations are not ideal because of the potential for movement of spores from fungicide-treated plots (interplot interference). Leaves were collected by cooperators and shipped to the Long Island Horticultural Research Laboratory (LIHRL), where sensitivity assays were conducted. Most of the 39 collections received were 19 to 45 leaves from a field. Spores were examined for fibrosin bodies to confirm the identity of these isolates as *S. fuliginea* (17). *Erysiphe cichoracearum* DC., another cucurbit powdery mildew fungus, occurs rarely (17,18).

Fungicide sensitivity assay. When possible, discrete colonies were selected from source leaves to minimize the chance of mixtures. Usually one colony per leaf was selected. Using an eyelash affixed to a disposable pipette, conidia were transferred to cotyledon disks of summer squash cv. Seneca Prolific on 2% water agar in petri dishes. All isolates were grown on these disks to obtain inoculum for the fungicide sensitivity assay. Cultures were incubated at 23°C/19°C (day/night) with a 12-h photoperiod.

Reference isolates of *S. fuliginea* known to be sensitive and resistant to both fungicides were included in each assay. These isolates were collected from a fungicide-treated research plot at the LIHRL in September 1990. They were maintained on detached, fungicide-free leaves in petri dishes. Conidia were transferred to new leaves every 2 to 4 weeks. The fungicide-resistant isolate was able to grow on leaf disks treated with triadimefon (active ingredient in Bayleton) at 200 µg/ml or benomyl (active ingredient in Benlate) at 200 µg/ml.

Fungicide sensitivity was tested on cotyledon leaf disks from fungicide-treated summer squash seedlings. The fungicide concentrations used were modified during the course of this study partly based on results obtained from assays. Triadimefon (formulated technical grade Bayleton 50DF, Bayer Corporation, Kansas City, MO) was dissolved in water and used at 0, 3.1, 5 or 6.3, 12.5, 25, and 50 µg/ml (3.1, 12.5, and 25 µg/ml were not used in all tests). Blank formulation for Bayleton 50DF (Bayer) was used to adjust all triadimefon solutions, including 0 µg/ml, such that they contained inert ingredients of the Bayleton 50DF formulation at 50 µg/ml. Benomyl, formulated as Benlate 50DF (E. I. DuPont de Nemours, Wilmington, DE), was used at 200 µg/ml. Propiconazole, formulated as Tilt 3.6E (Ciba-Geigy Corporation, Greensboro, NC), was used at 0.005, 0.01, 0.05, 0.5, 5, and 50 µg/ml. (In 1993, 0.005 and 50 µg/ml were discontinued, and in 1994, 0.01 µg/ml was discontinued.) Myclobutanil (RH-3866 technical fungicide, Rohm and Haas Company, Philadelphia, PA) was used at 0.001, 0.01, 0.1, 1, 2, and 20 µg/ml in 1993 (0.001 and 0.01 µg/ml were discontinued after several assays in 1993) and at 0.2, 2, and 20 µg/ml in 1994. RH-3866 was dissolved in acetone and methanol; dilutions were made using 1:1:2 acetone:methanol:water. Water was used for the no fungicide control (0 µg/ml) for benomyl, myclobutanil, and propiconazole because blank formulations were not available. Seedlings with expanded cotyledons (about 2 weeks after seeding) were sprayed with fungicide solutions using a DeVilbiss Model 152 atomizer (DeVilbiss Health Care, Inc., Somerset, PA) operated at 20 psi, air-dried, and 81 mm^2 disks were cut from cotyledons with a cork borer. Four disks treated with the same fungicide concentration were placed together on water agar in a single compartment of a petri dish with four sections or in a small (60 × 15 mm) petri dish. Propiconazole applied at 50 µg/ml does not have a sufficient vapor effect to cause a detectable impact on the growth of *S. fuliginea* on adjacent nontreated disks (M. T. McGrath and H. Staniszewska, *unpublished*). Vapor effect was not a concern with the other fungicides. Disks were inoculated about 1 day after treatment. Approximately three to five conidial chains (15 to 25 conidia) of each isolate were transferred to the center of each of the disks.

Quantity (percentage of leaf disk covered with mycelium) and quality of fungal growth were evaluated after incubation for 12 days using a dissecting microscope at 10.5× magnification. A higher magnification (63×) was used to find the inoculum and to see mycelium when growth was arrested. The following scale was used to rate quality: 0 = no growth (presence of inoculum was noted); 1 = very little mycelial growth; 2 = little mycelial growth with a few conidiophores; 3 = fairly good mycelial growth with scattered conidiophores; and 4 = good mycelial growth with good sporulation. Isolates were classified based on the highest fungicide concentration tolerated. An isolate was considered to be tolerant of a fungicide concentration when it produced conidiophores (rating of 2 to 4) on at least two of the four treated disks. Isolates were considered to be resistant if they were able to grow and produce conidiophores (quality rating of 2 to 4) on leaf disks treated with benomyl at 200 µg/ml or triadimefon at 50 µg/ml.

Statistical analysis. Spearman rank correlation coefficients were calculated using Minitab (Macintosh version release 8) to determine whether sensitivity to any of these fungicides was correlated (49). Ranks were assigned based on the highest fungicide concentration tolerated. Number of ranks for each fungicide varied with the

Table 1. Fungicide sensitivity of *Sphaerotheca fuliginea* isolates obtained from major cucurbit production areas in the United States in 1992 and 1993

State	Management[a]	Crop	No. of isolates	Date collected	Isolates able to grow on fungicide-treated leaf tissue (%) No fungic.	Benomyl (200)[b,c]	Triadimefon (50)[c]	Myclobutanil (2-20)	Propiconazole (0.5-5)
AZ	R; No Fungicide	Cantaloupe	29	6/7/93	100	0	69	86	Not tested
CA[d]	C; No Fungicide	Cantaloupe	13	5/19/93	100	0	69	77	92
CA[d]	C; Triadimefon ?	Cantaloupe	10	5/24/93	100	0	90	100	100
CA[d]	C; Triadimefon ?	Cucumber	4	6/1/93	100	0	100	100	Not tested
CA[d]	C; Triadimefon ?	Cucumber	3	6/7/93	100	0	67	100	Not tested
CA[d]	C; Triadimefon ?	Melon	8	6/7/93	100	0	75	100	Not tested
FL	C; Triadimefon-4X	Summer squash	36	12/16/92	100	3	81	Not tested	Not tested
FL	C; No Triadimefon/Benomyl	Summer squash	33	4/7/93	100	6	73	Not tested	Not tested
GA	R; No Triadimefon/Benomyl	Summer squash	38	7/6/93	100	0	16	24	29
MI	C; No Triadimefon/Benomyl	Pumpkin	41	9/16&23/93	100	7	2	5	10
NC	C; No Triadimefon/Benomyl	Melon	11	8/18/93	100	9	18	18	36
NY[e]	C; No Fungicide	Butternut squash	13	9/7&13/93	100	0	0	0	0
NY[e]	C; Triadimefon-3X/Benomyl-4X	Pumpkin	22	9/7&13/93	100	96	100	100	100
NY[e]	R; No Fungicide	Butternut squash	8	8/2/93	100	100	100	100	100
NY[e]	R; Triadimefon-2X	Pumpkin	17	8/2/93	100	100	100	100	100
VA	C; Triadimefon-1X	Summer squash	19	8/9/93	100	0	47	68	63
VA	C; Triadimefon-1X	Zucchini squash	18	8/9/93	100	0	6	72	72
VA	R; No Fungicide	Pumpkin	13	8/17/93	100	0	38	46	46
VA	R; Triadimefon-2X/Benomyl-2X	Pumpkin	15	8/17/93	100	0	60	67	67

[a] Management: C = commercial field, R = research plots, #X = number of applications of triadimefon, benomyl, or myclobutanil used.
[b] Fungicide concentration is expressed in µg of active ingredient per ml. The highest concentration used for triadimefon was 50 µg/ml; in previous tests, isolates able to tolerate this concentration also tolerated 200 µg/ml, which is above the solubility point for this material in water.
[c] Isolates able to tolerate these concentrations are considered to be resistant to the fungicide.
[d] Samples collected in May came from the Imperial Valley, and samples collected in June came from San Diego Co. ? = fungicide use records were not available, but triadimefon probably was applied. Triadimefon was applied to 50% of Imperial Valley's melon acreage, the main cucurbit crop, while benomyl was applied to 26% of the acreage (1993 annual pesticide use report).
[e] The commercial fields were in Eden, NY, and the research plots were in Freeville, NY.

concentrations tested. Ranks used for triadimefon were 1 for 0 and 3.1 µg/ml, 2 for 5 to 25 µg/ml, and 3 for 50 and 100 µg/ml. Ranks used for myclobutanil were 1 for 0 and 0.01 µg/ml, 2 for 0.1 and 0.2 µg/ml, 3 for 1 and 2 µg/ml, and 4 for 20 µg/ml. Ranks used for propiconazole were 1 for 0 and 0.01 µg/ml, 2 for 0.05 µg/ml, 3 for 0.5 µg/ml, and 4 for 5 µg/ml. Ranks used for benomyl were 1 for 0 µg/ml and 2 for 200 µg/ml. In order to obtain some idea of what the significance of the calculated coefficients might be, the procedure for calculating confidence limits for Pearson's correlation coefficients (49) was used, since there is not an exact way to do this for Spearman rank correlation coefficients.

RESULTS AND DISCUSSION

Source of isolates. *S. fuliginea* isolates were collected between 16 December 1992 and 19 September 1994 in nine states that include important cucurbit production areas in the western, central, and eastern portions of the United States (Tables 1 and 2). Isolates were from 13 fields that had been treated with triadimefon, one field that had been treated with myclobutanil (Experimental Use Permit 707-EUP-122), 19 non-triadimefon-treated fields, and four fields that probably had been treated with triadimefon. All 755 isolates tested were *S. fuliginea,* based on the presence of fibrosin bodies (17). In 1993 and 1994, triadimefon applied on a preventive schedule was the standard commercial practice for managing cucurbit powdery mildew in most of the United States. However, triadimefon usage was beginning to decline in 1994 because of recent performance problems.

Benomyl and triadimefon sensitivities. Isolates resistant to benomyl, to triadimefon, or to both were detected in all nine states in treated and nontreated fields (Tables 1 and 2). Isolates were considered to be resistant if they were able to tolerate benomyl at 200 µg/ml or triadimefon at 50 µg/ml. Previously, isolates able to tolerate these concentrations were found in fields where inadequate control was obtained with these fungicides (29,32). Resistant isolates were found in 18 of 19 fields that had not been treated with these fungicides and in 17 of 18 treated fields. Resistance to benomyl was less common than resistance to triadimefon. Benomyl-resistant isolates were found in eight of 19 nontreated fields in the current study, whereas triadimefon-resistant isolates were found in 18 fields. The proportion of resistant isolates obtained from a nontreated commercial field was as high as 25% for benomyl and 95% for triadimefon. In five of these fields, more than 50% of the isolates were triadimefon-resistant. There was no evident difference in growth between fungicide-sensitive and -resistant isolates in the absence of fungicide.

Sensitivity in treated fields. Applications of benomyl and/or triadimefon seemed to have an impact on fungicide resistance during a single growing season. The frequency of triadimefon-resistant isolates was higher in treated research plots of pumpkin in Virginia compared with the nontreated plots for this experiment (Table 1), and the frequency increased from 26 to 91% in a commercial field in Homestead, Florida, following four applications of myclobutanil (Table 2). The frequency of resistant isolates detected in the field treated once with triadimefon in Lantana, Florida, was lower than in three nontreated fields in Homestead, Florida (3% versus 12 to 26%, respectively; Table 2); however, the Lantana field was treated at least 4 weeks before isolates were collected, and triadimefon has not been applied often by growers in this area. Although benomyl was not used in the Lantana field where 9% of the isolates were benomyl-resistant, benomyl was applied at least four times for another problem by one grower located only about 6 to 7 km away. Benomyl and triadimefon resistance developed on a commercial farm in New York during the 1994 growing season, based on a comparison of the sensitivities of isolates collected on 1 August and 19 September (Table 2). Two triadimefon-treated squash fields in Virginia in 1993 with predominantly sensitive strains had been treated only once, only 13 days before samples were collected. The difference in frequency of resistant isolates may reflect the fact that although these fields were adjacent, one was closer to an older planting that had been treated with triadimefon previously. This treatment may have selected resistant strains that spread from the older to the younger planting. These observations on the apparent impact of fungicide usage on selection of resistant strains support previous results in which *S. fuliginea* populations were monitored over a growing season in treated and nontreated research plots and commercial fields (29).

Sensitivity in nontreated fields. Resistant strains were detected in nontreated fields. The occurrence of resistant strains

Table 2. Fungicide sensitivity of *Sphaerotheca fuliginea* isolates obtained from major cucurbit production areas in the United States in 1993 and 1994

					Isolates able to grow on fungicide-treated leaf tissue (%)				
State	Management[a]	Crop	No. of isolates	Date collected	No fungic.	Benomyl (200)[b,c]	Triadimefon (50)[c]	Myclobutanil (2-20)	Propiconazole (0.5-5)
CA	C; No Triadimefon/Benomyl	Zucchini squash	22	4/11/94	100	0	32	41	67
CA	C; No Triadimefon/Benomyl	Crenshaw melon	20	4/11/94	100	0	95	95	100
CA	C; No Triadimefon/Benomyl	Squash	23	5/10/94	100	0	4	100	Not tested
CA	C; No Triadimefon/Benomyl	Cantaloupe	4	5/10/94	100	0	60	40	Not tested
CA	C; No Fungicide	Zucchini squash	31	7/19/94	100	3	13	68	Not tested
FL	C; Not treated yet	Summer squash	44	12/8/93	100	25	20	68	30
FL	C; Not treated yet	Summer squash	25	3/24/94	100	16	12	60	80
FL	C; Not treated yet	Summer squash	27	3/24/94	100	7	26	7	96
FL	C; Myclobutanil-4X[d]	Summer squash	35	4/20/94	100	100	91	97	Not tested
FL	C; Triadimefon-1X	Summer squash	33	5/6/94	100	9	3	3	Not tested
GA	C; No Fungicide	Summer squash	23	5/27/94	100	9	17	91	Not tested
NY	C; Triadimefon-2X/Benomyl-2X	Buttercup squash	9	8/1/94	100	0	0	0	Not tested
NY	C; Triadimefon-4X/Benomyl-2X[d]	Pumpkin	11	9/19/94	100	60	73	100	Not tested
NY	R; Not treated yet	Summer squash	25	7/27/94	100	0	12	... [e]	Not tested
NY	R; Triadimefon-2X	Summer squash	14	8/31/94	100	100	100	100	Not tested
NY	R; Not treated yet	Pumpkin	23	8/6/94	100	0	39	Not tested	Not tested
NY	R; Triadimefon-1X	Pumpkin	8	8/19/94	100	100	100	100	Not tested
TX	C; Triadimefon-1X	Melon	23	5/30/94	100	0	0	17	Not tested
TX	R; Triadimefon-3X	Melon	14	5/30/94	100	0	71	100	Not tested
VA	C; No Fungicide	Butternut squash	38	8/9/94	100	0	58	76	Not tested

[a] Management: C = commercial field, R = research plots, #X = number of applications of triadimefon, benomyl, or myclobutanil used.
[b] Fungicide concentration is expressed in µg of active ingredient per ml. The highest concentration used for triadimefon was 50 µg/ml; in previous tests, isolates able to tolerate this concentration also tolerated 200 µg/ml, which is above the solubility point for this material in water.
[c] Isolates able to tolerate these concentrations are considered to be resistant to the fungicide.
[d] Same field or farm as previous entry in table.
[e] Twenty of 24 isolates grew at 0.2 µg/ml, the only concentration tested for 22 of these isolates. Two isolates tested at 2 µg/ml grew at this concentration.

at detectable levels in nontreated fields suggests that these strains are able to compete with sensitive strains in the absence of triadimefon or benomyl during a growing season. The frequency of fungicide-resistant isolates detected in some nontreated fields may have been high because of movement into these fields of resistant strains selected in nearby treated fields. This is a feasible explanation for the high percentage of triadimefon-resistant isolates detected in research fields in Arizona, New York, and Virginia and commercial fields in California and Florida in 1993 (Table 1). The nontreated research plots in Arizona were part of a fungicide efficacy trial in which about one-sixth of the field was treated with triadimefon, 25 and 10 days before the isolates were collected. The chlorothalonil-treated commercial field in North Carolina was within 16 km of fields that had been treated with triadimefon and benomyl. In contrast, only fungicide-sensitive isolates were found in the nontreated commercial field in New York, although it was located only about 1 km downwind from the fungicide-treated commercial field where only fungicide-resistant isolates were found. One possible explanation for this observation is that fungicide-sensitive strains were sufficiently numerous in the nontreated field when the influx of fungicide-resistant strains occurred that the population structure was not changed significantly.

Triadimefon resistance. Considering the widespread occurrence of triadimefon-resistant strains documented through this study, triadimefon resistance is the most feasible explanation for recent reports of reduced efficacy. Reduced efficacy of triadimefon compared with other fungicides and with previous performance was detected through replicated research experiments in New Jersey in 1986 (14), in Arizona in 1988 (25), in Michigan in 1989 (51), in Ohio in 1990 (41), in New York in 1990 (55), in North Carolina in 1992 (47), in Oklahoma in 1992 (5), in Tennessee in 1992 (3), and in California in 1993 (48). Commercial growers also have reported reduced efficacy.

Benomyl resistance. Benomyl resistance probably developed throughout the United States before this study, since this is the most feasible explanation for reduced efficacy documented in fungicide trials. Field testing of benomyl by researchers at public institutions was initiated in 1967. Other fungicides provided significantly better suppression of *S. fuliginea* than did benomyl, often in contrast with previous years, during fungicide efficacy trials in several states beginning in 1970. Reduced efficacy of benomyl was first reported in New York in 1970 (43), in California in 1970 (37), in Kentucky in 1973 (11), in New Jersey in 1974 (23), in North Carolina in 1974 (13), in Florida in 1977 (8), in Virginia in 1979 when used with chlorothalonil (1) and in 1981 when used alone (2), in Indiana in 1986 (21), and in Oklahoma in 1992 (5). Benomyl used alone failed to control *S. fuliginea* in Kentucky in 1973 (11), in North Carolina in 1974 (13), in Florida in 1979 (9), in New Jersey in 1980 (24), and in Indiana in 1988 (22). Commercial use of benzimidazole fungicides declined after these reports. There are few published reports on benomyl resistance in the United States. Benomyl-resistant isolates were detected in a commercial field as well as in research fields in New York in 1968 before this fungicide was registered for commercial use on cucurbits (44). They were detected again in New York in 1991 and 1992 (29) and in Michigan in 1991 (31).

Based on the low frequency of benomyl-resistant isolates detected during this study, resistance to benzimidazole fungicides apparently declined with the reduced use that was triggered by decreased efficacy in the 1970s. This decline in resistance occurred although benomyl-resistant strains appear to be competitive with sensitive strains during a growing season since they were detected in nontreated fields. Only 6% of 444 isolates from nontreated fields in the United States were resistant to benomyl. A higher frequency of resistant strains prior to treatment probably would be needed to have a control failure with benomyl. Benomyl was effective in fungicide efficacy trials in 1994 in Arizona (26), New Jersey (15), Ohio (40), and Oklahoma (4). Surprisingly, although benomyl was not effective in California in 1970 and 1971 (37), and although Imperial Valley growers have been using benomyl since about 1984 in combination with triadimefon due to reduced efficacy of triadimefon (G. Holmes, *personal communication*), only one benomyl-resistant isolate was detected among 138 isolates obtained for the present study from 10 fields in California in 1993 to 1994. This isolate came from Santa Maria County in July 1994. In a laboratory competition study with resistant and sensitive *S. fuliginea* isolates, the frequency of benomyl-resistant isolates declined in the absence of benomyl from 99% to almost zero in six generations (55). In contrast, *S. fuliginea* strains with resistance to benzimidazole fungicides persisted at high frequency under greenhouse conditions in the Netherlands, where the frequency of resistant isolates was still at 100% more than 10 years after these fungicides were withdrawn for control of cucurbit powdery mildew (42). In contrast with our findings with *S. fuliginea* under field conditions, resistant strains of *Botrytis* were found to be widespread in Alsace, France, 10 years after benzimidazole fungicide usage stopped (50).

Myclobutanil and propiconazole sensitivities. Sensitivities to the three DMI fungicides were correlated positively, whereas sensitivity to benomyl was not correlated with sensitivity to triadimefon, myclobutanil, or propiconazole based on Spearman rank correlation coefficients calculated for the highest fungicide concentration tolerated (Table 3). Ninety-one percent of isolates that tolerated propiconazole at 5 μg/ml were resistant to triadimefon (92% of isolates tested in 1993 and 85% of isolates in 1994) (Fig. 1). Ninety-four percent of isolates that tolerated myclobutanil at 20 μg/ml were resistant to triadimefon (94% of isolates tested in 1993 and 95% of isolates in 1994). In contrast, of the 134 isolates that were unable to grow on leaf disks treated with propiconazole at 0.5 μg/ml, only three were resistant to triadimefon. Furthermore, of 272 isolates that were unable to grow on leaf disks treated with myclobutanil at 2 μg/ml, only 16 were resistant to triadimefon. Genetic variation in fungicide sensitivity in one field ranged from a very sensitive isolate (unable to grow well at concentrations above 3 μg/ml [triadimefon], 0.1 μg/ml [myclobutanil], and 0.005 μg/ml [propiconazole]) to an insensitive isolate (able to tolerate 50 μg/ml [triadimefon], 20 μg/ml [myclobutanil], and 5 μg/ml [propiconazole]), which is a range of 16-fold for triadimefon, 200-fold for myclo-

Table 3. Spearman rank correlation coefficients for the highest concentrations of four fungicides tolerated by *Sphaerotheca fuliginea* isolates obtained from major cucurbit production areas in the United States in 1992 to 1994 and assayed on fungicide-treated leaf tissue

Collection period / Fungicide	Benomyl	Triadimefon	Myclobutanil
Dec. 1992 and 1993			
Triadimefon	0.418 (0.30,0.52)[a]		
Myclobutanil	0.489 (0.38,0.58)	0.755 (0.69,0.81)	
Propiconazole	0.389 (0.27,0.50)	0.763 (0.70,0.81)	0.762 (0.70,0.81)
Dec. 1993 and 1994			
Triadimefon	0.305 (0.18,0.42)		
Myclobutanil	0.163 (0.03,0.29)	0.590 (0.50,0.67)	
Propiconazole	−0.379 (−0.26,−0.49)	0.467 (0.36,0.56)	0.453 (0.34,0.55)
1992 to 1994			
Triadimefon	0.339 (0.25,0.42)		
Myclobutanil	0.336 (0.24,0.42)	0.685 (0.63,0.73)	
Propiconazole	0.243 (0.15,0.33)	0.709 (0.66,0.75)	0.696 (0.64,0.74)

[a] Confidence limits ($P = 0.99$). These are not exact. They are included to provide some indication of which coefficients might be significantly different.

butanil, and 1,000-fold for propiconazole. Correlated sensitivity (also called cross resistance or cross sensitivity) among DMI fungicides has been documented for *S. fuliginea* isolates from outside North America (12,19,35,36) and for other powdery mildew fungi (7,34). With most fungi, correlated sensitivity extends to all DMIs (6).

Most triadimefon-sensitive *S. fuliginea* isolates in the United States were not able to tolerate concentrations above 1.0 μg/ml (myclobutanil) and 0.5 μg/ml (propiconazole) (Fig. 1). The highest concentration of myclobutanil tolerated by triadimefon-sensitive isolates was 0.1 to 1.0 μg/ml for 46% of the isolates in 1993 and 54% of the isolates in 1994; 2 μg/ml was the highest concentration tolerated by 20 and 44% of the isolates in 1993 and 1994, respectively. Only 1.5% of 403 triadimefon-sensitive isolates tested were able to tolerate myclobutanil at 20 μg/ml. The highest concentration of propiconazole tolerated by triadimefon-sensitive isolates was 0.05 μg/ml for 44% of the isolates (60% in 1993 and 20% in 1994) and 0.5 μg/ml for 46% of the isolates (36% in 1993 and 78% in 1994). Only 3% of 256 triadimefon-sensitive isolates tested were able to tolerate propiconazole at 5 μg/ml.

Ineffective control with myclobutanil or propiconazole would not be predicted to occur within the next few years based on the level of insensitivity to these fungicides of triadimefon-resistant *S. fuliginea* isolates and on the performance of these fungicides in field trials where the efficacy of triadimefon was reduced. During a previous study, 79% of the triadimefon-resistant isolates able to tolerate 100 μg/ml were also able to tolerate 200 μg/ml, and 75% of these isolates did not exhibit a reduction in quantity of fungal growth at 200 μg/ml compared with lower concentrations (29). In contrast, most of the 87 isolates able to tolerate propiconazole at 5 μg/ml and most of the 98 isolates able to tolerate myclobutanil at 20 μg/ml exhibited a reduction in growth at these concentrations compared with lower concentrations. Following a shift in the *S. fuliginea* population to reduced sensitivity to all three fungicides, efficacy of triadimefon declined, whereas both propiconazole and myclobutanil remained highly effective (32). Powdery mildew was controlled effectively by myclobutanil but not by triadimefon in several other fungicide efficacy trials (3,14,25,47,51). Four applications of myclobutanil to a commercial field in Florida in 1994 resulted in an increase in the frequency of isolates with reduced sensitivity to myclobutanil (Table 2); however, none of the isolates were able to tolerate a higher concentration of myclobutanil (80 μg/ml) than isolates obtained from triadimefon-treated fields, and powdery mildew was controlled effectively (S. Crane, *personal communication*).

Predictions of future control failures with propiconazole or myclobutanil cannot be made with any certainty. It is not possible to predict whether *S. fuliginea* strains can develop that are able to tolerate higher concentrations of these fungicides than strains currently present in the United States. Recently introduced DMIs have higher activity than the first DMIs (including triadimefon) (6). This may account for the fact that newer DMIs have remained highly effective against some cereal powdery mildews (6). After a decade of widespread use of triadimenol and propiconazole to control wheat powdery mildew in Europe, *Erysiphe graminis* DC. f. sp. *tritici* Ém. Marchal has different resistance levels toward these materials: in contrast to triadimenol (maximum mean resistance factor value of 133 in 1993), the level of resistance to propiconazole (maximum value of 26) is similar or only slightly higher than the resistance level to recently introduced fungicides (10). Similarly, the resistance factor was found to be always greater for triadimefon than for propiconazole during a study of this pathogen in Japan (34). However, myclobutanil was not effective in a fungicide efficacy trial in Indiana in 1994 (R. Latin, *personal communication*). To preserve the usefulness of these fungicides as long as possible, fungicide resistance management strategies should be implemented immediately if these or other new DMIs are registered in the future.

Managing powdery mildew and fungicide resistance. Despite the occurrence of resistance, fungicides can still contribute positively to disease management (53). DMI fungicides have continued to be effective for managing grape powdery mildew (*Uncinula necator* (Schwein.) Burrill) in most situations in Europe since resistance was first detected in 1988 (52). When the initial frequency of resistant isolates was less than 40%, triadimefon suppressed powdery mildew in pumpkin early in the epidemic during the most critical time for yield protection (28–30). Although benomyl-resistant strains probably occurred naturally in New York before benomyl was applied to cucurbits, based on detection of resistant isolates in a commercial field in 1968 (44), this fungicide controlled powdery mildew significantly in fungicide efficacy trials compared with the nontreated control at least through 1974 and provided excellent control in 1972 and

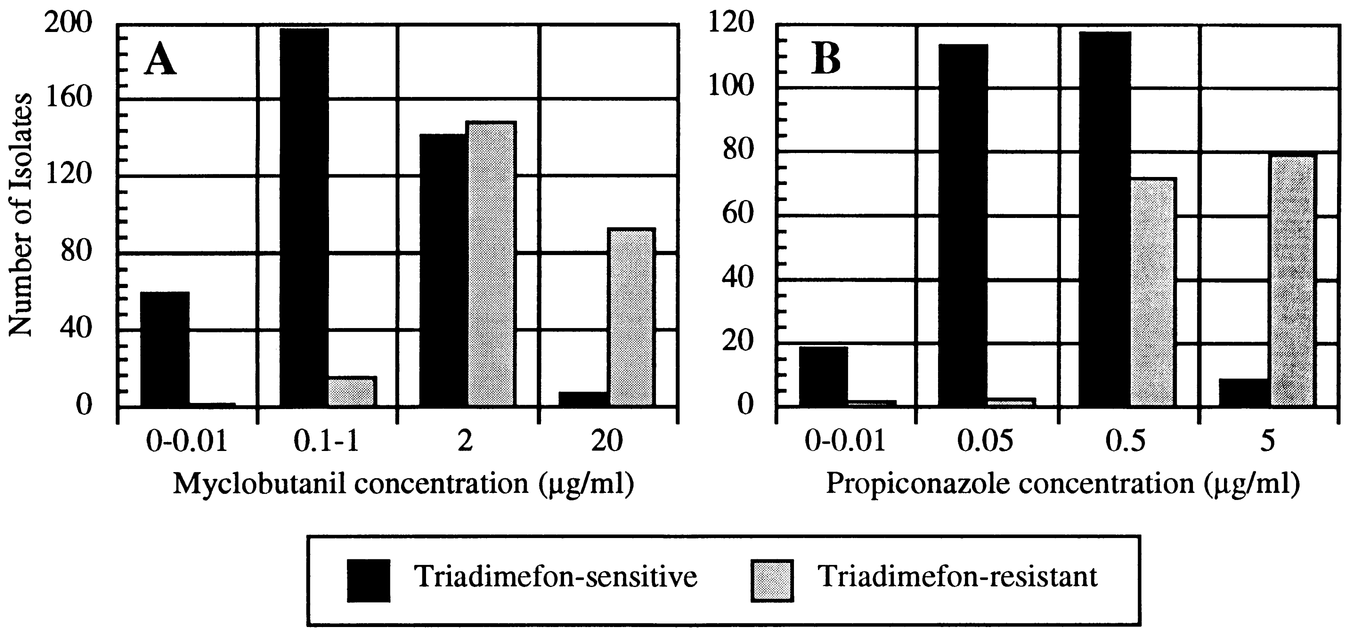

Fig. 1. Highest concentration of **(A)** myclobutanil and **(B)** propiconazole tolerated by triadimefon-sensitive isolates of *Sphaerotheca fuliginea* and isolates resistant to triadimefon at 50 μg/ml from California, Florida, Georgia, Michigan, North Carolina, New York, and Virginia in 1993 and 1994.

1974 when resistant isolates were not detected (39,45). Although benomyl-resistant strains of the grape powdery mildew fungus are common in commercial vineyards, control provided by benomyl wasn't significantly different from that provided by myclobutanil in 1992 (38).

The following recommendations for managing powdery mildew and fungicide resistance are based on results from this and previous studies on cucurbit powdery mildew as well as on published recommendations for other diseases (16,20). Managing resistance is important because systemic fungicides are extremely valuable tools for managing powdery mildew due to the difficulty of adequately covering abaxial leaf surfaces of cucurbit plants with a protectant fungicide using conventional sprayers. Integrated disease management programs with multiple control tactics are expected to be more stable than single-component programs (46). Disease severity should be reduced by nonchemical means such as growing resistant or less susceptible cultivars and physically separating successive plantings. Early plantings should be plowed under immediately after the last harvest.

DMI, benzimidazole, and other systemic fungicides prone to resistance development should be applied only a limited number of times during a season, only mixed with multisite protectant fungicides, and only early in disease development when they are expected to be most effective. Disease control early in crop development has the greatest impact on yield. Multisite protectant fungicides should be used alone during less critical times. Leaf coverage with protectant fungicides should be maximized by using application equipment and settings that deliver spray material throughout the canopy, especially to abaxial leaf surfaces. Even though resistance to triadimefon occurs throughout the United States, triadimefon is expected to be at least somewhat effective against powdery mildew because often there is a low frequency of triadimefon-resistant isolates at the onset of disease development in an area. At least one application of benomyl also is expected to be effective, based on the low frequency of benomyl-resistant isolates detected during this study. This situation could change in the future if benomyl use increases for powdery mildew or for other diseases; therefore, continued monitoring of fungicide sensitivity is needed. Subsequent applications of triadimefon or of benomyl are expected to be progressively less effective due to selection of resistant strains. More than one application per season of triadimefon or of benomyl may not be warranted because selection of resistant strains will result in decreasing disease suppression. A fungicide program consisting of protectant materials plus triadimefon and benomyl, applied together or in alternation, is expected to slow resistance development within a field, but not prevent it, compared with using one of these systemic fungicides alone. This program will not prevent resistance from developing because isolates resistant to both fungicides were found previously (29) and during this study. Systemic fungicides registered in the future should be used in place of triadimefon or benomyl. The ideal fungicide program for managing powdery mildew and fungicide resistance is multisite protectant fungicides plus at least two systemic fungicides to which *S. fuliginea* has not developed resistance and does not exhibit correlated sensitivity. Applying fungicides within a chemical class is not recommended because of correlated sensitivity.

Although triadimefon-resistant isolates apparently are not able to survive well between seasons since fungicide-sensitive isolates predominate at the start of the season, triadimefon-resistant isolates apparently are fit since they have been found with sensitive isolates in fields that were not treated with fungicides. The implication of this for managing powdery mildew and fungicide resistance is that triadimefon or benomyl applied to early plantings of cucurbits most likely will result in selection of resistant isolates, which could be sources of inoculum for later plantings. Others have recommended that only full rates be used because selection of less sensitive strains can be increased by using reduced and split fungicide applications due to continuous selection pressure at low dose (20,46,52). This tactic should be effective when the fungal population includes strains capable of tolerating reduced rates but not full rates.

ACKNOWLEDGMENTS

This work was supported in part by funds from Rohm and Haas Company, Philadelphia, PA, and Ciba-Geigy Corporation, Greensboro, NC, and funds allocated by the New York State College of Agriculture and Life Sciences through Hatch Project 153445. Appreciation is extended to Katherine Sheehan for technical assistance; to Bayer Corporation, Kansas City, MO, E. I. DuPont de Nemours, Wilmington, DE, and Robson Seed Farms Corporation, Hall, New York, for providing fungicides and seed used in these studies; to K. L. Pernezny, S. E. Crane, M. E. Matheron, A. O. Paulus, K. Mayberry, D. Kelley, D. R. Sumner, J. D. Gay, M. E. Miller, R. E. Baldwin, E. Rutkowski, M. Hausbeck, C. W. Averre, and T. A. Zitter for collecting leaves with powdery mildew; and to D. R. Sumner, R. E. Baldwin, T. A. Zitter, K. L. Reynolds, G. Holmes, and P. A. Arneson for comments on the manuscript.

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