Increased Resistance to Triadimefon and to Benomyl in *Sphaerotheca fuliginea* Populations Following Fungicide Usage over One Season

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ABSTRACT

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Fungicide sensitivity of *Sphaerotheca fuliginea* was monitored during powdery mildew epidemics in research plots and production fields of pumpkin. Benomyl-resistant isolates (insensitive to 200 µg/ml) were detected in all populations before benomyl was used. The only triadimefon-resistant isolate (insensitive to 100 µg/ml) detected prior to treatment was near triadimefon-treated plants. Populations in nontreated fields did not change appreciably. Fungicide application had a large impact on fungicide resistance. Proportion of resistant isolates in research plots treated four times with triadimefon plus chlorothalonil shifted from 0 to 81% for triadimefon and from 30 to 69% for benomyl between 15 August and 19 September 1991; proportion shifted from 3 to 100% for triadimefon and from 10 to 44% for benomyl between 14 August and 17 September 1992. The shift occurred rapidly: proportion of resistant isolates shifted within 2 weeks from 0 to 96% for triadimefon and from 10 to 74% for benomyl following two applications of triadimefon in a commercial field in August 1992. Most isolates (87%) from fungicide-treated fields tolerated triadimefon at 100 or 200 µg/ml. These were considered triadimefon-resistant because fungicide performance declined during September. However, control was commercially acceptable. Of 122 isolates collected from fungicide-treated pumpkin, one was benomyl-resistant, triadimefon-sensitive; 20 were benomyl-sensitive, triadimefon-resistant; six were sensitive to both fungicides; and 95 were resistant to both with 77 of these insensitive to triadimefon at 200 µg/ml. In contrast, 57 of 92 isolates from nontreated populations were sensitive to both fungicides, none were resistant to both, and 32 were resistant to benomyl.

Additional keywords: benzimidazole, cucurbits, DMI, EBI, SBI

Fungicide resistance may become a major factor with which to contend in managing *Sphaerotheca fuliginea* (Schlechtend.:Fr.) Pollacci, the primary causal agent of powdery mildew of cucurbits in the United States. Powdery mildew is an important foliar disease of cucurbits, occurring every year in most production areas of the United States. Its management is required to avoid a reduction in yield or market quality for most crops (12). 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.). Systemic fungicides are required to suppress disease development on abaxial leaf surfaces, where powdery mildew develops most extensively. However, resistance has developed to systemic fungicides (5,7,17,18, 21,23), since they tend to be site-specific inhibitors. In contrast, most protectant fungicides affect several metabolic processes. Selection pressure for development of fungicide resistance in *S. fuliginea* populations may be particularly strong because systemic fungicides function alone on abaxial leaf surfaces, even when applied with a protectant fungicide (a standard strategy to reduce the rate of selection for resistance). This happens because it is difficult to thoroughly cover abaxial surfaces with a protectant fungicide using a conventional boom sprayer, especially for vining cucurbit crops. Only three systemic fungicides in two classes are registered for use to manage powdery mildew in the United States: benomyl and thiophanate-methyl, benzimidazoles, and triadimefon, a demethylation inhibitor (DMI). DMIs are a subgroup in the ergosterol biosynthesis inhibitor (EBI or SBI) group of fungicides. Many commercial growers use triadimefon, and the benzimidazoles are used less extensively for powdery mildew; therefore, the fungus probably is exposed to the same systemic fungicides throughout the United States. If the pathogen is dispersed northward each year from southern production areas, then resistant isolates selected in one area could serve as initial inoculum in another area.

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Resistance of *S. fuliginea* to both classes of fungicides has been documented outside of North America. In Australia, resistance to benzimidazole fungicides was recognized just 3 years after their introduction in 1970 (21). Reduced efficacy of fenarimol, which is in the pyrimidine group of SBI fungicides, was reported on field-grown cucumbers in Israel in 1982, only 4 years after product introduction and 2 years after use became intensive (5). Sensitivity testing revealed resistance to several SBI fungicides in field- and greenhouse-grown cucurbits in Israel, Greece, and Spain (5). Reduced sensitivity to triadimefon was detected in a Japanese greenhouse after 2 years of use that provided successful control (18). Strains of *S. fuliginea* resistant to an SBI fungicide often are resistant to other fungicides in this group (5,7,17,18).

Fungicide resistance of *S. fuliginea* in the United States has not been examined since 1969, when benomyl-resistant isolates were first documented (23). Decline in benomyl effectiveness was noted in California in 1970 and 1971 (19) and in Florida in 1983 (24).

Research on fungicide resistance was initiated following detection of resistance to triadimefon and to benomyl in *S. fuliginea* isolates collected in September 1990 from research plots of pumpkins at the Long Island Horticultural Research Laboratory (LIHRL). Objectives of the research conducted in 1991 and 1992 were to determine sensitivity to triadimefon and to benomyl of *S. fuliginea* isolates at the start of disease development and to determine the impact of using these fungicides on resistance during a growing season. These fungicides were selected because both are used by commercial growers in the United States. Research plots and commercial fields were used. Preliminary reports of part of this work have been made (13,15).

MATERIALS AND METHODS

Powdery mildew populations. Fungicide sensitivity was monitored in powdery mildew populations in fungicide-treated commercial pumpkin production fields, in fields of organically grown pumpkin, and in research plots of nontreated and chlorothalonil plus triadimefon treated pumpkin at the LIHRL. The research plots were 6.9 × 13.7 m and contained 80 plants in four rows (9,14). Thirty *S. fuliginea* isolates were obtained from leaves collected from each population at the start and at the end of the powdery mildew epidemics. Each field and each treatment group, which consisted of four replicated research plots, was considered to be a separate population. Collections could not be made from all fields on the same date because time and space constraints permitted only one fungicide sensitivity assay to be performed per week. Spores were examined for fibrosin bodies to confirm the identity of these isolates as *S. fuliginea* (6). *Erysiphe cichoracearum* DC ex Merat also causes cucurbit powdery mildew but lacks these structures (4).

1991. Isolates were collected from a fungicide-treated commercial pumpkin production field on 29 July and 26 September and from a field of organically grown pumpkin on 1 August and 12 September. For the experimental field, leaves with powdery mildew were collected on 15 August from nontreated interplot areas; and on 19 September, four isolates were collected from each of four plots that were nontreated or treated with triadimefon and chlorothalonil. Interplot areas were selected to avoid disturbing plants and powdery mildew development in plots at the start of epidemics.

Research plots were sprayed with triadimefon (Bayleton 50DF, 0.14 kg a.i./ha, Bayer Corporation, Kansas City, MO) plus chlorothalonil (Bravo 720, 2.81 kg a.i./ha, ISK Biosciences Corp., Mentor, OH) on 23 July, 8 and 23 August, and 7 September, and with chlorothalonil alone on 31 July, 16 and 30 August, and 16 September. These are the highest rates labeled for the United States. Chlorothalonil is a broad-spectrum contact fungicide with activity against *S. fuliginea* (8). These fungicide-treated plots and the nontreated plots also were used for another experiment, which included eight other fungicide treatment groups (9). Treatments were initiated after 15 August for the eight other fungicide treatment groups in this experiment. Benomyl (Benlate 50DF, E. I. duPont de Nemours, Wilmington, DE) was applied twice to one of these treatment groups. A randomized block design with four replications was used for the 10 treatment groups. Triadimefon was applied to the commercial pumpkin crop on 7 and 22 August and 9 September. Thiophanate-methyl (Topsin M, Elf-Atochem North America, Philadelphia, PA) was applied on 15 and 29 July. All applications included chlorothalonil.

1992. Thirty isolates were collected from a field of organically grown pumpkins on 30 July and 10 September. For the experimental field, leaves with powdery mildew were collected on 14 August from nontreated interplot areas and on 17 September from treated and nontreated plots. Only 10 powdery mildew colonies could be found on 6 August in the fungicide-treated commercial pumpkin field. An additional collection was made from this field 2 weeks later on 21 August. The final collection from this field was made on 24 September. These populations were in different locations from the 1991 populations.

Chlorothalonil plus triadimefon–treated research plots were sprayed with both fungicides on 21 July, 6 and 21 August, and 5 September and with chlorothalonil alone on 30 July and 13 and 27 August (14). Triadimefon was applied twice, on 5 and 13 August, to the commercial pumpkin crop before the collection on 21 August. Benomyl was applied on 20 August and 10 September. All four applications included chlorothalonil.

Additional powdery mildew populations were examined in September to evaluate the antiresistance strategy of using two systemic fungicides with different modes of action. Isolates were collected on 17 September from a commercial pumpkin crop that had been treated routinely with thiophanate-methyl and/or triadimefon plus chlorothalonil or a copper fungicide. Isolates were collected on 18 September from research plots treated with triadimefon (Bayleton 50DF, 0.07 kg a.i./ha), benomyl (Benlate 50WP, 0.14 kg a.i./ha) plus chlorothalonil (Bravo 720, 2.81 kg a.i./ha) on 6 August, 22 August, and 5 September. On 23 September, eight isolates were obtained from research plots in Freeville, New York, that had been treated with triadimefon and benomyl (26) to determine if fungicide resistance occurs in upstate New York.

Fungicide sensitivity assay. Discrete colonies were selected on pumpkin leaves to minimize the chance of mixtures. Using an eyelash or hair affixed to a disposable pipette, conidia were transferred to cotyledon disks (81 mm^2) of growth chamber–grown summer squash cv. Seneca Prolific on 2% water agar in 9 cm^2 petri dishes. All isolates were grown on these disks for about 13 days to obtain inoculum for the fungicide sensitivity assay. Cultures were incubated at 23°C/19°C (day/night) with a 12-h photoperiod at 9,688 lux.

Reference isolates of *S. fuliginea* known to be moderately 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 (10). 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 at 200 µg/ml or benomyl at 200 µg/ml. It was not exposed to fungicides during the present study. The fungicide-sensitive isolate was able to grow on leaf disks treated with triadimefon at 12.5 µg/ml (also 25 µg/ml in some tests); thus it was moderately sensitive to triadimefon compared to other isolates.

Fungicide sensitivity was tested on cotyledon leaf disks from fungicide-treated summer squash seedlings. Isolates were tested with triadimefon at 0, 12.5, 25, 37.5, 50, 100, and 200 µg/ml (technical grade Bayleton 50DF, Bayer Corporation, Kansas City, MO), and with benomyl at 200 µg/ml (formulated as Benlate 50WP). Blank formulation for Bayleton 50DF was used to adjust all triadimefon solutions, including 0 µg/ml, such that they contained 200 µg/ml of inert ingredients of the Bayleton 50DF formulation. Seedlings were dipped in fungicide solutions for 3 s and air-dried overnight. Then 81 mm^2 disks were cut with a cork borer, and three or four disks treated with the same fungicide concentration were placed together on water agar in a compartment of a 9 cm^2 petri dish with four sections. Approximately three to five conidial chains (total of 15 to 25 conidia) of each isolate were transferred to the center of each disk. After incubation for 12 days at 23°C/19°C (day/night) with a 12-h photoperiod, quantity (percent of leaf disk covered with mycelium) and quality (presence of conidia and sporulation density) of fungal growth were evaluated using a dissecting microscope at 10.5× magnification or at 63× when there was little or no growth to confirm the presence of inoculum.

Data analyses. Dose-response curves were examined to determine if there was a consistent trend in response among isolates, thus allowing calculation of EC_{50} values (the effective concentration at which fungal growth is reduced by 50%). Graphs with untransformed data, logistic transformation for quantity of fungal growth, or logarithmic transformation for fungicide concentration were examined.

The quantities of fungal growth on disks treated with triadimefon at 0 µg/ml for fungicide-sensitive and -resistant isolates were compared to determine if fungicide-resistant isolates grew slower than fungicide-sensitive isolates. Analysis of variance was done with data for isolates from research plots at the end of the season, because there was a similar number of fungicide-sensitive and -resistant isolates in these two tests.

Chi-square tests (2 × 2) were conducted to determine whether there were significant differences in the proportion of resistant isolates between fungicide-treated and nontreated populations for production fields and for experimental fields.

Powdery mildew development. Disease development was monitored in the fungicide-treated commercial field in 1992 to assess if any changes in fungicide sensitivity were associated with an increase in powdery mildew severity. Adaxial and abaxial leaf surfaces of young, mid-aged, and old leaves were examined. Powdery mildew colonies were counted; percent leaf area covered with colonies was estimated when colonies could not be counted accurately because they had coalesced and were too numerous. An average severity value was calculated for all leaf types. Disease development also was monitored in research plots as part of another experiment involving these plots in this field (9,14).

RESULTS

General assay results. Most isolates either grew well (abundant mycelium with good sporulation) or grew poorly (no or little mycelial growth without sporulation) at each fungicide concentration. Since triadimefon and other DMI fungicides interfere with germ-tube elongation and have little effect on spore germination (2), it is not surprising that sensitive isolates were able to begin to grow. At each concentration tolerated, including 0 μg/ml, variation occurred in the percent disk area covered by mycelium for the four replicate disks (Fig. 1). When there was abundant fungal growth on only two or three of the four disks, which occurred occasionally, the disks with little or no growth were disregarded, and the isolate was considered insensitive or tolerant of this concentration. This probably reflects variation in leaf tissue or quality of inoculum, because similar results occurred with nontreated disks (M. T. McGrath, *unpublished*). Another feasible explanation is variation in quantity of fungicide deposited. On the other hand, when an isolate grew on only one disk at a particular fungicide concentration, this disk was disregarded and the isolate was considered sensitive. Only one isolate did not grow at any concentration of triadimefon, including 0 μg/ml, perhaps because it was sensitive to the inert ingredients in the Bayleton 50DF formulation or the spores transferred were not viable. It was not included in the tabulation of results.

The relationship between concentration of triadimefon and percent disk area covered by mycelium was not sufficiently consistent among the isolates examined to permit calculating EC_{50} values (Fig. 1). Many isolates exhibited a nonlinear relationship that was not linearized with a logarithmic or logistic transformation. Seventy-five percent of the isolates that were insensitive to 200 μg/ml did not exhibit a reduction in growth at this concentration (Fig. 1A). Percent disk area covered by mycelium gradually declined with increasing concentration for 25% of the isolates (Fig. 1B). Many isolates (44%) that were moderately resistant to triadimefon grew equally well on disks below a particular concentration and were unable to grow on disks treated with higher concentrations of triadimefon (Fig. 1C). Isolates were classified by the highest triadimefon concentration tolerated rather than by EC_{50} value.

Most (56%) of the 406 isolates (398 from Table 1 plus eight from Freeville, New York) tested during this study were only able to grow on disks treated with 0 μg/ml triadimefon (Table 1). Many (137 isolates) were able to grow on disks treated with triadimefon at 100 or 200 μg/ml, and thus they were considered highly resistant. Solubility of triadimefon in water at 20°C is 64 μg/ml; however, more active ingredient does go into suspension at higher concentrations according to the manufacturer and based on the growth response of *S. fuliginea*. Isolates were considered moderately sensitive to triadimefon if they were able to grow and produce spores on disks treated with triadimefon at 12.5 or 25 μg/ml. One reference isolate and 26 of 406 isolates fell in this category. Only 17 isolates were able to grow on disks treated with triadimefon at 37.5 or 50 μg/ml but not at 100 μg/ml; they were considered moderately resistant. The fungicide-resistant reference isolate has maintained its level of resistance to triadimefon (200 μg/ml) and to benomyl (200 μg/ml) in the absence of selection pressure from these fungicides for over 3 years.

Fungicide-resistant isolates did not grow more slowly than fungicide-sensitive isolates on 0 μg/ml triadimefon-treated leaf disks. There was no difference in percent leaf disk covered with fungal growth between fungicide-resistant and -sensitive isolates obtained from the research plots at the end of the season in 1991 ($P = 0.19$). Quantity of growth was greater for fungicide-resistant isolates (mean disk coverage with powdery mildew of 93%) than for fungicide-sensitive isolates (73%) obtained from the research plots in 1992 ($P = 0.0001$).

1991. Most (65%) of the 88 isolates collected from three fields in July or August at the start of the powdery mildew epidemic were sensitive to triadimefon and benomyl. None of these 88 isolates were resistant to triadimefon (Table 2). Fourteen were able to grow on 12.5 μg/ml triadimefon disks; however, this probably reflects an anomaly in this particular assay because the sensitive reference isolate was able to grow at a higher concentration than in other assays. Thirty-one (35%) were able to grow in the presence of benomyl at 200 μg/ml. Although benomyl had been used only in the fungicide-treated commercial

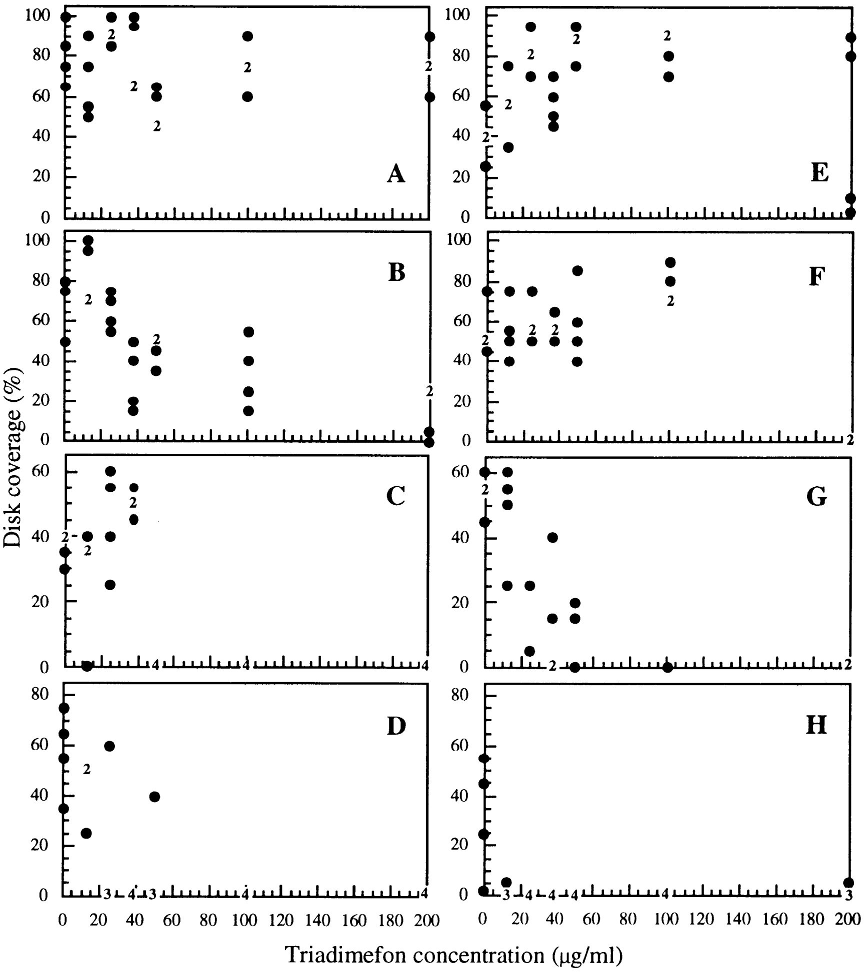

Fig. 1. Growth of eight *Sphaerotheca fuliginea* isolates on triadimefon-treated leaf disks showing the range in fungicide sensitivity and dose-response curves observed. These isolates were collected **(A and B)** on 21 August 1992 or **(C to H)** on 26 September 1991 from fungicide-treated pumpkin. **(A)**, **(B)**, **(E)**, and **(F)** are highly resistant; **(C)**, **(D)**, and **(G)** are moderately resistant; and **(H)** is moderately sensitive. Numbers on the graphs indicate the number of leaf disks with the same amount of growth.

field, benomyl-resistant isolates were found in all fields.

Management of powdery mildew had an impact on fungicide resistance. Where fungicides were not used for disease management, fungicide sensitivity changed little from August to September (Table 2). At the end of the epidemic in September, 40 isolates from powdery mildew populations in nontreated pumpkins were sensitive to triadimefon and six isolates were moderately sensitive (Table 1). The proportion of isolates that were resistant to benomyl seemed to have decreased (Table 2). Five isolates from the research field and eight from the commercial field grew on only one of four benomyl-treated disks. If these isolates are classified as resistant rather than sensitive, then 44 and 53% of isolates were benomyl-resistant from these fields, respectively. In sharp contrast, 81% of isolates collected in September from research plots, which had been sprayed four times with triadimefon, and 89% of isolates from a commercial field, which had been sprayed three times with triadimefon, were moderately or highly resistant to triadimefon. Most of these resistant isolates (30 of 38) grew even with triadimefon at 200 µg/ml, the highest concentration used. Five did not grow at concentrations above 100 µg/ml, two did not grow above 50 µg/ml, and 1 did not grow above 37.5 µg/ml (Table 1). Five of six triadimefon-sensitive isolates grew on disks treated with 12.5 µg/ml. Percentage of isolates resistant to benomyl in the commercial field shifted from 17% on July 29 to 89% on September 26. This may have occurred because thiophanate-methyl, which has the same mode of action as benomyl, was applied in late July and in mid-September. Although neither benomyl nor thiophanate-methyl was used in the fungicide-treated research plots, the percentage of the population that was resistant to benomyl shifted from 30 to 69%. Three isolates from the commercial field that were sensitive to benomyl were sensitive, moderately sensitive, and moderately resistant to triadimefon. Three of five isolates from fungicide-treated research plots that were sensitive to benomyl were moderately sensitive to triadimefon. The proportion of resistant isolates in fungicide-treated populations was significantly higher than the proportion in nontreated populations in September for both production and experimental fields for both triadimefon and benomyl (Table 2).

1992. Thirty-six of the 69 isolates (52%) collected in July or August at the start of powdery mildew development were sensitive to both triadimefon and benomyl (Table 3). Five isolates were moderately sensitive to triadimefon (able to grow on 12.5 µg/ml–treated disks) (Table 1); one of these was benomyl resistant. One isolate was highly resistant to triadimefon (able to grow on 100 µg/ml–treated disks) and sensitive to benomyl. This isolate was obtained from a nontreated interplot area in the research field near two plots that had been treated twice with triadimefon prior to collection. This isolate probably was selected in a triadimefon-treated plot, and conidia were dispersed into the interplot area. Another isolate grew on 37.5 µg/ml–treated disks; however, the moderately sensitive reference isolate also grew at this concentration only in this particular test, which suggests that concentrations may not have been correct. Twenty-seven of 69 isolates were benomyl resistant. Benomyl had not been used in any fields prior to the first collection.

Fungicide sensitivity did not change appreciably in powdery mildew populations in nontreated pumpkin from August to September (Table 3). The only triadimefon-resistant isolates found in nontreated pumpkins were in research plots in September 1992. These three isolates were moderately resistant to triadimefon and sensitive to benomyl. They may have originated from triadimefon-treated plots, since seven of nine fungicide treatments in this research field included triadimefon.

Fungicide usage resulted in selection of powdery mildew isolates with decreased sensitivity to triadimefon and to benomyl (Table 3). Selection of fungicide-resistant strains occurred extremely fast in fungicide-treated commercial pumpkins. After only 2 weeks and two applications of triadimefon, resistance to triadimefon shifted from 0% of the population to 96%, and resistance to benomyl shifted from 10 to 74%. In fungicide-treated research plots, resistance to triadimefon shifted from 3 to 100% of the population between 14 August

Table 1. Highest triadimefon concentration tolerated by *Sphaerotheca fuliginea* isolates collected at the start of powdery mildew development in late July to early August and by isolates collected near the end of the growing season in late September from five commercial fields and three research fields on Long Island, New York

			Triadimefon concentration (µg/ml)						
			Sensitive	Moderately sensitive		Moderately resistant		Highly resistant	
Field description	Collection date	isolates	0	12.5	25	37.5	50	100	200
Nontreated commercial	2 Aug. 1991	28	28[a]	0	0	0	0	0	0
	12 Sep. 1991	30	25	4	1	0	0	0	0
Nontreated commercial	30 July 1992	30	28	1	1	0	0	0	0
	10 Sep. 1992	30	24	6	0	0	0	0	0
Fungicide-treated commercial[b]	29 July 1991	30	30	0	0	0	0	0	0
	26 Sep. 1991	28	1	2	0	1	0	4	20
Fungicide-treated commercial[c]	6 Aug. 1992	10	9	1	0	0	0	0	0
	21 Aug. 1992	27	0	0	1	2	0	2	22
	24 Sep. 1992	30	0	0	0	1	2	3	24
Fungicide-treated commercial[d]	17 Sep. 1992	18	0	0	0	0	2	3	13
Research plots[e]	15 Aug. 1991	30	30	0	0	0	0	0	0
Nontreated research plots	19 Sep. 1991	16	15	1	0	0	0	0	0
Fungicide-treated research plots[f]	19 Sep. 1991	16	0	3	0	0	2	1	10
Research plots[e]	14 Aug. 1992	29	25	3	0	0	0	1	0
Nontreated research plots	17 Sep. 1992	16	11	1	1	2	1	0	0
Fungicide-treated research plots[g]	17 Sep. 1992	16	0	0	0	1	2	7	6
Fungicide-treated research plots[h]	18 Sep. 1992	14	0	0	0	0	1	6	7
All fields	all	398	226	22	4	7	10	27	102

[a] Number of isolates for which the triadimefon concentration listed was the highest tolerated.
[b] Triadimefon was applied on 7 and 22 August and 9 September.
[c] Triadimefon was applied on 5 and 13 August.
[d] Triadimefon was applied on 6, 21, and 31 August and 10 September.
[e] Isolates were collected from interplot areas of the research fields in August.
[f] Triadimefon was applied on a preventive schedule on 23 July, 8 and 23 August, and 7 September.
[g] Triadimefon was applied on a preventive schedule on 21 July, 6 and 21 August, and 5 September.
[h] Triadimefon was applied on 6 and 22 August and 5 September.

and 17 September 1992, and resistance to benomyl shifted from 10 to 44% even though benomyl was not applied in these plots. The proportion of triadimefon-resistant isolates in fungicide-treated populations was significantly higher than the proportion in nontreated populations in September for both production and experimental fields; the proportion of benomyl-resistant isolates in fungicide-treated populations was significantly higher only in the production field (Table 3).

Powdery mildew development was suppressed by fungicide treatment for most of the growing season. In the fungicide-treated commercial field, average powdery mildew severity on adaxial and on abaxial leaf surfaces was 0.0 and 0.2%, respectively, on 25 August and 5 and 13.3% on 9 September. Control of powdery mildew with fungicides was considered commercially acceptable because disease development was suppressed until September and leaves were in good condition during most of the fruit development and maturation period.

Fungicide-resistant isolates also were found in other fields in September 1992 (Table 1). Of 14 isolates tested from research plots treated with triadimefon, benomyl, and chlorothalonil, eight were resistant to both triadimefon and benomyl, and six were resistant to triadimefon but sensitive to benomyl. Of 18 isolates tested from a commercial field treated with triadimefon and thiophanate-methyl, 16 were resistant to both triadimefon and benomyl, and two were resistant to triadimefon but sensitive to benomyl. All Freeville isolates were highly resistant to both fungicides.

DISCUSSION

Applying the systemic fungicide triadimefon with or without benomyl to pumpkin during one growing season was associated with a substantial increase in the proportion of the powdery mildew population that was resistant to triadimefon and to benomyl in both replicated research plots and commercial production fields. This population shift in fungicide-treated fields is not likely due to selection pressure from another factor, such as change in environmental or host-related conditions, because fungicide sensitivity did not change appreciably for populations that were not treated with fungicides, most notably nontreated plots. The proportion of the *S. fuliginea* population with resistance to triadimefon increased from 0–3% to 81–100% in 34 to 59 days after three to four triadimefon applications in research plots and commercial fields in 1991 and 1992. Most of the change in fungicide sensitivity was found to have occurred within the first 2 weeks after two applications of triadimefon. Fungicide resistance is not a unique problem to Long Island, New York, since only resistant isolates were detected in Freeville, New York. Changes in fungicide sensitivity following treatment have been reported previously. The proportion of the *S. fuliginea* population with resistance to triadimefon (25 μg/ml) increased from 34 to 93% after five weekly spray applications in an unreplicated trial in Australia (17). Resistance to the SBI fungicides fenarimol and imazalil increased over a 2-year period in *S. fuliginea* populations in greenhouses in the Netherlands (22).

Most triadimefon-resistant isolates (110 of 154) grew even with triadimefon at 200 μg/ml, the highest concentration used in this study. Although EC_{50} values were not calculated during the present study because the variability in the assay did not allow a good fit of a model to the dose response, most of these isolates would have an EC_{50} value greater than 200 μg/ml. Thus, they are similar to resistant isolates detected in Australia (17). The EC_{50} values of six *S. fuliginea* isolates tested in Australia were for triadimefon greater than 200 μg/ml for two isolates, 20 μg/ml for one isolate, and less than 10 μg/ml for three isolates. In contrast, relatively low EC_{50} values of 0.7 μg/ml and 1.0 μg/ml were reported for a fungicide-resistant isolate of *S. fuliginea* tested in the United Kingdom (7) and an isolate tested in Japan (18), respectively.

Table 2. Effect of powdery mildew management on fungicide resistance in 1991

	Isolates fungicide resistant (% (no.))[a]			
	Triadimefon		Benomyl	
	July/Aug.	Sep.	July/Aug.	Sep.
Commercial fields				
Nontreated[b]	0 (0/28)	0 (0/30)	61 (17/28)	27 (8/30)
Fungicide-treated[c]	0 (0/30)	89 (25/28)	17 (5/30)	89 (25/28)
Chi-square[d]		47.08	11.94	23.16
Research fields				
Nontreated[e]	0 (0/30)	0 (0/16)	30 (9/30)	12 (2/16)
Fungicide-treated[e]		81 (13/16)		69 (11/16)
Chi-square[d]		21.90		10.49

[a] Frequency of *Sphaerotheca fuliginea* isolates that were moderately or highly resistant to triadimefon (able to grow on 37.5 to 200 μg a.i./ml) or resistant to benomyl (able to grow on 200 μg a.i./ml). In parentheses, number of resistant isolates/total number of isolates tested.

[b] Isolates were collected on 1 August and 12 September.

[c] Isolates were collected on 29 July and 26 September. Triadimefon was applied on 7 and 22 August and 9 September. Thiophanate-methyl was applied on 15 and 29 July.

[d] Chi-square value from comparison of number of sensitive and resistant isolates in the two preceding samples in the column. Values greater than 3.84 indicate that the proportions for the two samples are significantly different at $P = 0.05$.

[e] Isolates were collected on 15 August and 19 September. Triadimefon was applied to the fungicide-treated plots on 23 July, 7 and 23 August, and 7 September.

Table 3. Effect of powdery mildew management on fungicide resistance in 1992

	Isolates fungicide resistant (% (no.))[a]					
	Triadimefon			Benomyl		
	July/Aug.	Aug.	Sep.	July/Aug.	Aug.	Sep.
Commercial fields						
Nontreated[b]	0 (0/30)		0 (0/30)	77 (23/30)		60 (18/30)
Fungicide-treated[c]	0 (0/10)	96 (26/27)	100 (30/30)	10 (1/10)	74 (20/27)	97 (29/30)
Chi-square[d]			60.00	0.83		11.88
Research fields						
Nontreated[e]	3 (1/29)		19 (3/16)	10 (3/29)		25 (4/16)
Fungicide-treated[e]			100 (16/16)			44 (7/16)
Chi-square[d]			21.90			1.25

[a] Frequency of *Sphaerotheca fuliginea* isolates that were moderately or highly resistant to triadimefon (able to grow on 37.5 to 200 μg a.i./ml) or resistant to benomyl (able to grow on 200 μg a.i./ml). In parentheses, number of resistant isolates/total number of isolates tested.

[b] Isolates were collected on 30 July and 10 September.

[c] Isolates were collected on 6 August, 21 August, and 24 September. Triadimefon was applied on 5 and 13 August. Benomyl was applied on 20 August and 10 September.

[d] Chi-square value from comparison of number of sensitive and resistant isolates in the two preceding samples in the column. Values greater than 3.84 indicate that the proportions for the two samples are significantly different at $P = 0.05$.

[e] Isolates were collected on 14 August and 17 September. Triadimefon was applied to the fungicide-treated plots on 21 July, 6 and 21 August, and 5 September.

Triadimefon-resistant isolates did not appear to be less fit then sensitive isolates. Quantity of growth on disks treated with triadimefon at 0 μg/ml was not less for resistant isolates than for sensitive isolates. Infection efficiency and ability to produce spores also seemed to be unaffected. Furthermore, the fungicide-resistant reference isolate used in the present study maintained its degree of resistance to triadimefon and to benomyl without exposure to these fungicides for over 3 years, which suggests that resistance to both fungicides is stable. The fitness components, latent period and sporulation, were not found to be correlated with resistance to SBI fungicides for *Pyrenophora teres* (20).

Most fungicide-resistant isolates were resistant to both triadimefon and benomyl. Of the 122 isolates collected in September 1991 and September 1992 from fungicide-treated research plots and commercial fields, 78% (95 isolates) were resistant to both fungicides. Only 20 of 115 triadimefon-resistant isolates were sensitive to benomyl; 11 of these were moderately resistant to triadimefon. Similarly, isolates were detected in Australia with multiple fungicide resistance encompassing the benzimidazole and SBI fungicide groups as well as the hydroxypyrimidine and organophosphate groups (17). In contrast, SBI-resistant isolates in Greece and Spain were sensitive to benomyl (5).

The frequency of isolates resistant to benomyl increased in three fields that had been sprayed with triadimefon but not with benomyl. These were research plots in both years and the commercial field in 1992 between the first and second collections. This increase cannot be ascribed to other selection factors because a similar increase did not occur in nontreated fields. Simultaneous development of resistance to both triadimefon and benomyl as a result of cross-resistance is not expected to occur when only one of these fungicides is used, because they have different modes of action and thus resistance to these fungicides would not be caused by the same genetic factor. The most feasible explanation is that most of the triadimefon-resistant isolates early in powdery mildew development on Long Island had multiple resistance because they were selected for in other fields where both triadimefon and benomyl were used. This explanation is supported by the fact that most triadimefon-resistant isolates detected in other populations (Long Island, 1993, and Florida, 1992 and 1993) were benomyl-sensitive (11,15). It also is possible that the apparent increase in frequency of benomyl-resistant isolates is not real and represents sampling error.

The change in the fungicide sensitivity of the *S. fuliginea* populations was associated with a decline in fungicide efficacy toward the end of the growing season, when powdery mildew would not be expected to have a significant impact on yield. Research plots treated with triadimefon plus chlorothalonil in 1992 had very low levels of powdery mildew from 28 July, when symptoms were observed first, through 9 September, which was 4 days after the third application (12). In these plots, the average powdery mildew severities on adaxial and on abaxial leaf surfaces were 0 and 2%, respectively, on 9 September and 0 and 35% on 18 September; in nontreated plots, severities were 73 and 83%, respectively, on 9 September. Disease development on abaxial leaf surfaces after 9 September may be due to triadimefon being ineffective because of fungicide resistance. Chlorothalonil continued to suppress *S. fuliginea* on adaxial leaf surfaces through 18 September in the plots treated with chlorothalonil alone (5%) or with chlorothalonil plus triadimefon (0%). Triadimefon applied alone suppressed powdery mildew effectively only through 1 September, which was 11 days after the second application of triadimefon. Average powdery mildew severities on adaxial and on abaxial leaf surfaces were 12 and 13%, respectively, on 9 September in these plots. Disease development apparently unsuppressed by triadimefon treatment late in the growing season probably did not have a yield impact because similar yield and fruit quality were obtained in 1993 from pumpkin treated with triadimefon or with another SBI fungicide that maintained effectiveness through the growing season (16).

However, control failure due to development of fungicide resistance is a potential problem. Disease control achieved with triadimefon was not adequate in an unreplicated trial in Australia (17). As compared with the present study, the proportion of the *S. fuliginea* population with resistance to triadimefon at the start of the experiment in Australia was high (34%), which may account for the differing results. Resistance to several SBI fungicides was associated with poor disease control when disease pressure was high and spray coverage was incomplete in field- and greenhouse-grown cucurbits in Israel, Greece, and Spain (5).

Management strategies for powdery mildew following development of fungicide resistance have been examined. Control was improved to acceptable levels by reducing spray intervals, increasing water volumes, and increasing fungicide dosages after fungicide resistance became a problem in field- and greenhouse-grown cucurbits in Israel, Greece, and Spain (5). Including other fungicides in the management program in a Netherlands greenhouse resulted in improved control compared with the previous year, when SBI treatments had not provided expected control and a reversal of the trend toward decreased fungicide sensitivity had occurred in the *S. fuliginea* population (22). Applying triadimefon with a protectant fungicide may slightly delay resistance development based on a comparison of powdery mildew development in research plots treated with triadimefon alone or with chlorothalonil (12). Applying triadimefon in combination with a protectant fungicide (chlorothalonil) and with another systemic fungicide possessing a different mode of action (benomyl or thiophanate-methyl) did not prove to be an effective antiresistance strategy when used in a commercial field and an experimental field on Long Island, based on fungicide sensitivity of the powdery mildew population. All 32 isolates collected from these two fields in mid-September 1992 were resistant to triadimefon; 75% (24 isolates) were benomyl resistant. Resistance to both fungicides was detected in 57% of the isolates from the research plots treated with both systemic fungicides and 44% of the isolates from the research plots treated with triadimefon. These research plots were in different fields. In Greece and Spain, where SBI-resistant isolates were found to be sensitive to benomyl, powdery mildew development in greenhouses was suppressed well with tank mix applications of nuarimol and benomyl or with applications of benomyl alone (5).

The current study provides valuable information for designing a management program. Triadimefon should be more effective than benomyl for managing powdery mildew, because only benomyl-resistant isolates were detected in all *S. fuliginea* populations prior to selection pressure from use of these fungicides. Triadimefon efficacy is expected to decline following treatment to a cucurbit crop due to selection of resistant isolates.

Based on results from this study, the following guidelines are suggested for managing cucurbit powdery mildew and fungicide resistance. Triadimefon should be applied once or twice with a protectant fungicide. Any subsequent applications should be only protectant fungicides. Systemic fungicides should only be used in combination with protectant fungicides. Additional applications of triadimefon may not be sufficiently effective. Adequate control is not expected with benomyl or thiophanate-methyl during most years in New York. However, a benzimidazole fungicide may be valuable used in a management program with triadimefon when the frequency of benomyl-resistant strains is initially low, as occurred in Florida in 1992 and on Long Island in 1993 (11). Limited use (one application per year) has been an effective strategy for ethirimol in Europe, presumably because ethirimol-sensitive forms of barley powdery mildew reinvade when residues decrease (1). Similarly, one application of dimethirimol at the most necessary time is recommended for cucumber powdery mildew (1). Successful management of phenylamide resistance and late blight of potatoes in the Netherlands has been achieved by applying metalaxyl a maximum of twice, only with a protectant fungicide and only in critical situations (25).

Triadimefon efficacy in the future is difficult to predict. Resistant isolates did not appear to be less fit than sensitive isolates based on growth under laboratory conditions. However, resistant isolates were not detected before triadimefon was applied in 1991 and 1992 despite the fact that 81 to 100% of the isolates were resistant after treatment. Other fitness components could be involved. Ethirimol-resistant *Erysiphe graminis* f. sp. *hordei* isolates grow and sporulate normally under laboratory conditions but do not compete well with sensitive types under field conditions (3). In contrast with the situation on Long Island, triadimefon-resistant isolates of *S. fuliginea* in Australia seem to be competitive with sensitive isolates, because they were detected at a high frequency (34%) before fungicides were applied and they were common in nontreated plots (17).

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LITERATURE CITED

1. Brent, K. J. 1982. Case study 4: Powdery mildews of barley and cucumber. Pages 219-230 in: Fungicide Resistance in Crop Protection. J. Dekker and S. G. Georgopoulos, eds. Centre for Agricultural Publishing and Documentation, Pudoc, Wageningen.
2. Fuchs, A., and Waard, M. A. 1982. Resistance to ergosterol-biosynthesis inhibitors. I. Chemistry and phenomenological aspects. Pages 71-86 in: Fungicide Resistance in Crop Protection. J. Dekker and S. G. Georgopoulos, eds. Centre for Agricultural Publishing and Documentation, Pudoc, Wageningen.
3. Hollomon, D. W. 1978. Competitive ability and ethirimol sensitivity in strains of barley powdery mildew. Ann. Appl. Biol. 90:195-204.
4. Homma, Y. 1937. Erysiphaceae of Japan. J. Fac. Agric. Hokkaido Univ. 38:183-461.
5. 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.
6. Kable, P. K., and Ballantyne, B. J. 1963. Observations on the cucurbit powdery mildew in the Ithaca district. Plant Dis. Rep. 47:482.
7. Kendall, S. J. 1986. Cross-resistance of triadimenol-resistant fungal isolates to other sterol C-14 demethylation inhibitor fungicides. Br. Crop Prot. Conf.-Pests Dis. 2:539-546.
8. Lewis, G. D. 1988. Acorn squash powdery mildew control, 1987. Fungic. Nematicide Tests 43:146.
9. McGrath, M. T. 1992. Efficacy of fungicides applied preventatively or based on disease occurrence for managing powdery mildew of pumpkins, 1991. Fungic. Nematicide Tests 47:124.
10. McGrath, M. T. 1994. Heterothallism in *Sphaerotheca fuliginea.* Mycologia 86:517-523.
11. McGrath, M. T. 1994. Implications of fungicide resistance for managing powdery mildew, now and in the future. Pages 77-80 in: New York State Vegetable Conference. New York State Vegetable Growers Association, Liverpool, NY.
12. McGrath, M. T. 1996. Successful management of *Sphaerotheca fuliginea* in pumpkin with triadimefon applications initiated after disease detection. Plant Dis. In press.
13. McGrath, M. T., and Ghemawat, M. S. 1992. Effective management of powdery mildew in pumpkin with triadimefon despite development of fungicide resistance following treatment. (Abstr.) Phytopathology 82:1157.
14. McGrath, M. T., Ghemawat, M. S., and Staniszewska, H. 1993. Efficacy of fungicides applied preventively or following disease detection for managing powdery mildew of pumpkin, 1992. Fungic. Nematicide Tests 48:173.
15. McGrath, M. T., and Staniszewska, H. 1993. Fungicide resistance in cucurbit powdery mildew: Effect of fungicide usage and impact on control. (Abstr.) Phytopathology 83:1389.
16. McGrath, M. T., and Staniszewska, H. 1994. Efficacy of fungicides applied preventively or following disease detection for managing powdery mildew of pumpkin, 1993. Fungic. Nematicide Tests 49:142.
17. O'Brien, R. G., Vawdrey, L. L., and Glass, R. J. 1988. Fungicide resistance in cucurbit powdery mildew (*Sphaerotheca fuliginea*) and its effect on field control. Aust. J. Exp. Agric. 28:417-423.
18. Ohtsuka, N., Sou, K., Amano, T., Ojima, M., Nakazawa, Y., and Yamada, Y. 1988. Decreased sensitivity of cucumber powdery mildew (*Sphaerotheca fuliginea*) to ergosterol biosynthesis inhibitors. Ann. Phytopathol. Soc. Jpn. 54:629-632.
19. Paulus, A. O., Nelson, J., Shibuya, F., Whitaker, T. W., House, J., Meister, H., and Bohn, G. W. 1972. Fungicides and methods of application for the control of cantaloupe powdery mildew. Plant Dis. Rep. 56:935-938.
20. Peever, T. L., and Milgroom, M. G. 1994. Lack of correlation between fitness and resistance to sterol biosynthesis-inhibiting fungicides in *Pyrenophora teres.* Phytopathology 84:515-519.
21. Peterson, R. A. 1973. Field resistance to benomyl in cucurbit powdery mildew. Aust. Plant Pathol. Soc. Newsl. 2:27-28.
22. Schepers, H. T. A. M. 1985. Changes during a three-year period in the sensitivity to ergosterol biosynthesis inhibitors of *Sphaerotheca fuliginea* in the Netherlands. Neth. J. Plant Pathol. 91:105-118.
23. Schroeder, W. T., and Provvidenti, R. 1969. Resistance to benomyl in powdery mildew of cucurbits. Plant Dis. Rep. 53:271-275.
24. Sonoda, R. M., and Pohronezny, K. 1983. Status and potential of pesticide resistance among fungal and bacterial pathogens of vegetable crops in Florida. Proc. Fla. State Hortic. Soc. 96:66-69.
25. Staub, T. 1991. Fungicide resistance: Practical experience with antiresistance strategies and the role of integrated use. Annu. Rev. Phytopathol. 29:421-442.
26. Zitter, T. A., and Hsu, L. 1993. Comparison of hydraulic and electrostatic sprayers for fungicide applications in winter squash, 1992. Fungic. Nematicide Tests 48:184.