Resistance of *Coccomyces hiemalis* to Benzimidazole Fungicides

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ABSTRACT

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Benomyl-resistant strains of the cherry leaf spot fungus (*Coccomyces hiemalis*) were detected in a sour cherry orchard in 1975, in another in 1976, and in three sweet and four sour cherry orchards and a fruit tree nursery in 1979. Sensitive isolates were strongly inhibited in a disk assay at 1.0 μg/ml benomyl; resistant strains were weakly inhibited at 800–1,000 μg/ml. Resistant strains developed in orchards where benomyl had been used exclusively for 3–5 yr and where mixtures of benomyl and nonsystemic fungicides had been applied after 1975, the year a resistant strain was first detected. The isolates were also resistant to carbendazim and thiophanate-methyl.

Additional key words: *Prunus avium, P. cerasus*

Cherry leaf spot, incited by the fungus *Coccomyces hiemalis* Higgins, commonly occurs on sour and sweet cherries in Michigan. The disease is particularly important economically considering the 4,553 ha (11,244 acres) of sweet cherries and 16,616 ha (41,028 acres) of sour cherries grown in the state (1). An annual program of four to six fungicide applications is used to prevent early defoliation of cherry trees from the disease.

In 1975, we detected benomyl-resistant *Monilinia fructicola* (Wint.) Honey on cherries in Michigan and stated, without presenting supporting data, that benomyl-resistant *C. hiemalis* was also identified (5). In 1979, leaf spot was a problem in some cherry orchards where benomyl was mixed or alternated with nonsystemic fungicides. Therefore, we sought to determine if benomyl-resistant strains of leaf spot were present in these orchards. In this paper we report the increase and distribution of resistant strains in the state. To our knowledge, benomyl-resistant *C. hiemalis* has not been detected in other cherry-growing areas.

MATERIALS AND METHODS

The techniques used to detect resistant strains were similar to those used for *Venturia inaequalis* (Cke.) Wint. (6,8). Leaf disks containing single lesions were cut with a number 3 cork borer from infected cherry leaves and streaked sequentially across divided petri dishes containing potato-dextrose agar (PDA; GIBCO Diagnostics, Madison, WI 53711) in one half and PDA supplemented with 25 μg/ml benomyl in the other. Fresh stock solutions of benomyl 50% a.i. WP were added to the media after autoclaving and cooling to about 60 C. About 30 disks, with no more than two disks per leaf, were cut from leaves taken from a composite sample of leaves collected in each orchard. Conidia that germinated and produced normal germ tube elongation on the benomyl-amended medium in about 24 hr were considered resistant.

Isolates for further study were obtained by transferring single spores from the PDA plates to 60-mm-diameter petri dishes containing PDA or lima bean agar (Difco Laboratories, Detroit, MI 48201). Conidia of each isolate were produced on lima bean agar slants as described by Magie (7). Resistance to other fungicides was determined by dipping sterilized 12.7-mm-diameter assay disks into solutions containing 0, 1, 10, 100, 500, and 1,000 μg/ml fungicide and by placing a saturated disk in the center of each petri dish seeded 5 hr earlier with 0.5 ml of conidial suspension adjusted to about 10^4 conidia/ml.

RESULTS AND DISCUSSION

Benomyl-resistant strains of the leaf spot fungus were detected in a sour cherry orchard in 1975, in another in 1976, and in three sweet and four sour cherry orchards in 1979. One of the sour cherry orchards identified in 1979 was a fungicide research orchard on the Michigan State University campus. Resistant strains were also detected on sour and sweet cherries in a fruit tree nursery in 1979.

Most of the orchards were heavily defoliated when the samples were taken and a high proportion of the leaf disks from these orchards yielded resistant spores. Sites with resistant strains occurred in each of the important cherry-growing areas of the state (Fig. 1). Five of the sites were in the counties surrounding Traverse City, where 78% of the sweet cherries and 49.5% of the sour cherries are grown in Michigan (1). In two cases, resistance was detected on adjacent farms, but the remaining locations were well separated from one another and the benomyl-resistant strains were considered geographically distinct.

In 1975, five single-spore isolates of putative resistant strains from one orchard were tested by using the disk assay, and no inhibition zones developed on media amended with benomyl at rates of 0.1, 1.0, 10, 20, 40, 60, 80, 100, 200, 400, and 600 μg/ml. Small zones of inhibition were noted with three isolates at 800 μg/ml and with all isolates at 1,000 μg/ml. Inhibition zones were produced with six sensitive isolates at 1.0 μg/ml. No inhibition zones were noted at 1,000 μg/ml with two resistant isolates from the orchard in 1976, although inhibited zones were noted at 1.0 μg/ml benomyl with three sensitive isolates from other orchards. All resistant strains sporulated in the presence of benomyl on artificial media.

In limited greenhouse trials, resistant isolates infected cherry leaves sprayed with benomyl 50% a.i. WP at 226.8

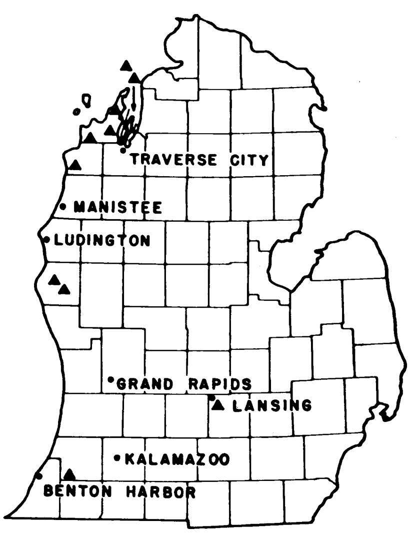

Fig. 1. Geographic distribution of benomyl-resistant *Coccomyces hiemalis* in Michigan. All but two locations were detected in 1979. (▲ = site of benomyl-resistant cherry leaf spot.)

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g/378.5 L of water, but sensitive isolates were controlled. In a field trial in 1976, benomyl 50% a.i. WP at 226.8 g/378.5 L failed to control leaf spot under light disease pressure (4), and 97% of the leaf disks from benomyl-sprayed trees yielded resistant conidia. Isolates resistant to benomyl were also resistant to carbendazim and thiophanate-methyl in vitro, which agrees with most reports of benomyl-resistant fungi. Fungicide concentrations to 1,000 μg/ml benomyl, carbendazim, and thiophanate-methyl did not result in inhibition zones with resistant isolates, but benomyl or carbendazim at 1.0 μg/ml or thiophanate-methyl at 10 μg/ml produced inhibition zones with sensitive isolates. Fungicides containing thiophanate-methyl or carbendazim have little merit for use on cherries in Michigan because of cross-resistance problems.

Table 1. Pattern of benomyl use in cherry orchards where resistant strains of *Coccomyces hiemalis* were detected

Orchard[a] Year	Total fungicide applications (no.)	Benomyl 50% a.i. WP Application (no.)	Benomyl 50% a.i. WP Rate[b] (g)	Fungicides (and rates) combined with benomyl[b]
Sour cherry, no. 1				
1975	7	5	150.2	None
1974	7	7	150.2	None
1973	6	6	150.2	None
1972	4	0	···	···
Sour cherry, no. 2[c]				
1979	3	3	226.8	None
1978	5	5	226.8	None
1977	6	6	170.1	None
1976	5	5	226.8	None
1975	5	5	226.8	None
Before 1975	···	0	···	···
Sour cherry, no. 3				
1979	10	6	113.4	Captan 50% a.i. WP 453.6 g
1978	6	2	113.4	Captan 50% a.i. WP 453.6 g
1977	7	5	113.4	Captan 50% a.i. WP 453.6 g
		1	113.4	Sulfur 95% a.i. WP 2,267.9 g
1976	7	4	226.8	None
1975	6	6	226.8	None
1973–1974	···	0	···	···
Sweet cherry, no. 4				
1979	6.5[d]	1.5	113.4	Sulfur 95% a.i. WP 1,360.7 g
1978	5	1	113.4	Sulfur 95% a.i. WP 1,360.7 g
1977	6	1	170.1	Sulfur 95% a.i. WP 1,360.7 g
1976	6	1	226.8	None
		0.5	226.8	Dodine 65% a.i. WP 113.4 g
		0.5	226.8	Sulfur 95% a.i. WP 1,360.7 g
1975	5.5	3	226.8	None
1974	6	0	···	···
1973	6.5	0.5	170.1	None
Sweet cherry, no. 5[e]				
1979	7	4	113.4	Captan 80% a.i. WP 340.2 g
		2	113.4	Sulfur (0.7 kg/L) 510.2 g
1978	6	4	113.4	Captan 80% a.i. WP 283.5 g
1977	5	1	113.4	Captan 80% a.i. WP 283.5 g
		1	113.4	Sulfur (0.7 kg/L) 340.2 g
1976	5	1	204.1	Sulfur (0.7 kg/L) 340.2 g
1975	5	3	170.1	None
Sour cherry, no. 6				
1979	7	2	113.4	Captan 80% a.i. WP 303.3 g
		3	113.4	Sulfur (0.7 kg/L) 680.4 g
1978	6	1	113.4	Diclone 50% a.i. WP 226.8 g
		1	113.4	Cyprex 65% a.i. WP 113.4 g
		1	113.4	Sulfur (0.7 kg/L) 510.2 g + Cyprex 65% a.i. WP 113.4 g
		1	113.4	Captan 80% a.i. WP 303.3 g
1977[f]	7	1	56.7	Superior oil 473.0 ml
		1	170.1	Diclone 50% a.i. WP 226.8 g
		1	170.1	Sulfur (0.7 kg/L) 452.5 g
		2	170.1	Captan 80% a.i. WP 303.3 g

[a] Percent of viable samples yielding spores resistant to benomyl in vitro ranged from 97 to 100% in the six orchards.

[b] Amount of fungicide formulation per 378.5 L (100 U.S. gal.) of water. Generally, the equivalent of 1,135.5 and 1,514.0 L (300 and 400 U.S. gal.) of dilute spray per acre are applied on sour and sweet cherries, respectively.

[c] Experimental orchard on the Michigan State University campus. No resistance was detected in 116 samples from two other experimental orchards with less exposure to benomyl.

[d] Sprays were applied on an alternate middle row system. Two half sprays were counted as a complete application.

[e] Resistant strains may have originated in an adjacent sour cherry orchard where benomyl-resistant leaf spot and brown rot fungi were detected. Spray records for the sour cherry orchard were not available.

[f] Benomyl 50% a.i. WP was also applied before 1977, but records are incomplete.

In examining fungicide histories for six problem orchards, we found that benomyl programs were applied in orchards 1 and 2 (Table 1) and in a sour cherry orchard where resistance was detected in 1976 (schedule not available). Resistant conidia were detected in the third year of exclusive benomyl use except in orchard 2, where only about 15% of the trees received a full benomyl program each year for 5 yr. In orchards 3, 4, 5, and 6, benomyl was sometimes alternated with nonsystemic fungicides until 1976 or 1977, when tank mixtures of benomyl and nonsystemic fungicides were applied after the bloom period of flower bud development. Nonbenzimidazole fungicides were used during bloom to control brown rot blossom blight and occasionally at other times.

Resistance developed in orchards 3, 4, 5, and 6 even though benomyl was frequently mixed and alternated with nonsystemic fungicides. Except for dodine, the fungicides mixed with benomyl are rated as weak for leaf spot control even when used at high concentrations (2,3). Benomyl was the principal fungicide used against leaf spot, and insufficient control was provided by the second fungicide to prevent a buildup of resistant strains. A high level of selection pressure may have been exerted on the pathogen population before mixed programs were initiated in these orchards.

Leaf spot resistance creates a major problem for Michigan cherry growers because alternative fungicides for combined brown rot and leaf spot control are less effective or more costly or require more frequent applications than benomyl. Captafol is a possible alternative on mechanically harvested sour cherries, but it has undesirable toxicological properties. Captan and sulfur are alternative fungicides for sweet cherries but are much less effective and have phytotoxicity and compatibility problems. With benomyl resistance, the choice of fungicides effective against either leaf spot or brown rot is severely limited until new fungicides are registered.

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