Cercospora beticola Tolerant to Triphenyltin Hydroxide

William M. Bugbee

U.S. Department of Agriculture,
Agricultural Research Service,
Northern Crop Science Laboratory
Fargo, ND 58105-5677

ABSTRACT

Three of five sugarbeet fields from two districts in Minnesota had unacceptable levels of Cercospora leaf spot after being treated with the maximum label amount of triphenyltin hydroxide (TPTH) in 1994. Strains of the fungus with varied levels of tolerance to TPTH were recovered from leaf spots. Mancozeb was effective against the tolerant strains. Strains with resistance to thiophanate methyl also were recovered.

Additional Key Words: Organic tin fungicides, systemic fungicides, protectant fungicides.

Triphenyltin fungicides (hydroxide, chloride, or acetate) are very effective against *Cercospora beticola* L. for the control of leaf spot on sugarbeet (*Beta vulgaris* L.) (Carlson, 1966; Finkner et al., 1966; Stallknecht and Calpouzos, 1968). Triphenyltin hydroxide (TPTH) is superior to copper and carbamate fungicides in toxicity and persistence on leaves (Stallknecht and Calpouzos, 1968) and has been used extensively in the Northern Plains of the United States where Cercospora leaf spot is a problem. TPTH usage increased dramatically after the rapid development of strains that became resistant to the benzimidazole class of fungicides in the early 1970s (Ruppel and Scott, 1974), and TPTH now is the primary fungicide for Cercospora leaf spot control on sugarbeets.

Strains of *C. beticola* tolerant to triphenyltin have been reported from Greece (Giannopolitis, 1978), Italy (Cerato, 1983), and Yugoslavia (Maric et al., 1984). Since 1986, field isolates of *C. beticola* have been tested in our laboratory for tolerance to TPTH with negative results. In 1994, fields in west-central and southern Minnesota had control of Cercospora leaf spot that was not as complete as had been experienced in the past. When conidia of *C. beticola* were transferred from leaf spots to media containing TPTH, the amount of growth indicated that the fungus had acquired tolerance to the fungicide. Evidence of tolerant strains is reported here.

MATERIALS AND METHODS

Technical grade TPTH, (97.1% a.i). was dissolved in acetone and added to potato-dextrose agar (PDA). In a test of the effect of autoclaving, TPTH was added at 0.125, 0.250, 0.500, or 1.000 μg ml^{-1} before autoclaving, or to molten (55° C) PDA after autoclaving. Three tin-tolerant strains were transferred to the culture media. The test was repeated once.

Technical grade 95.7% a.i. thiophanate-methyl (dimethyl [(1,2-phenylene)bis(iminocarbonothioyl)] = bis[carbamate] also was dissolved in acetone and added to PDA before autoclaving. In one test, thiophanate methyl was added before or after autoclaving to measure the effect on linear growth of the fungus.

Acetone alone was added to PDA controls. Lids were kept off poured culture plates for 30 min to allow dissipation of acetone in a laminar-flow transfer hood.

A commercial preparation of mancozeb (zinc ion complexed with manganous ethylenebis[dithiocarbamate]) (75% a.i.) was suspended in distilled water and added to 55° C PDA after autoclaving.

Streptomycin sulfate was added at 300 μg ml^{-1} to 55 C PDA after autoclaving to retard bacterial growth in all culture media that received conidia directly from leaf spots. Conidia of *C. beticola* were transferred from leaf spots to culture media with the aid of a dissecting microscope and glass needles. The glass needles were rinsed in 70% ethanol to reduce contamination. Leaves were placed in clear plastic moist chambers and incubated in the greenhouse overnight to induce sporulation in cases of scarce conidia. Leaves were preserved by drying overnight at 50° C.

Hyphal plugs, 4 mm in diameter, were removed with a cork borer from the margins of 6- to 10-day-old agar cultures of *C. beticola* and transferred to PDA amended with TPTH, mancozeb or both. The tin-tolerant strains of *C. beticola* were maintained on PDA with 0.125 μg ml^{-1} TPTH. Six plugs, one each of six isolates being tested, were placed in one square culture dish, 10 x 10 cm. Three of the isolates were from southern Minnesota and three were from west-central Minnesota. Colony diameters were measured after 6 days at 25° C. Treatments were in triplicate in a completely randomized design, and the test was repeated once. The means of growth responses of *C. beticola* to fungicide dosages was compared by regression analysis.

A collection of 57 randomly selected isolates from southern and west-central Minnesota were tested against four concentrations of TPTH for inhibition of linear growth in order to determine the prevalence of tolerance. Each isolate was cultured in triplicate, and colony diameters were measured after 6 days at 25° C. The effectiveness of the fungicides was expressed as percent inhibition compared to the control [(control-test/control) X 100].

RESULTS AND DISCUSSION

Autoclaving significantly ($P = 0.05$) reduced the toxicity of TPTH. Average inhibition of three tin-tolerant isolates grown on four concentrations of TPTH was 26% when TPTH was added before autoclaving and 46% when added after. Therefore, assays for tolerance were done on postautoclave-amended media in order to measure the full effect of the fungicide. Autoclaving increased the toxicity of thiophanate methyl as was reported for benomyl (Ogawa et al., 1979). When thiophanate methyl was added at 1 μg ml^{-1} before autoclaving, the average inhibition of the two test isolates was 93%, but only 16% when added after autoclaving. The effect of autoclaving on linear growth was not evident at 5 μg ml^{-1}; therefore, thiophanate methyl was added to PDA at 5 μg ml^{-1} before autoclaving in standard assays.

Isolates of *C. beticola* with tolerance to 5 $\mu g\ ml^{-1}$ TPTH were found in leaf samples from two of four problem fields in west-central Minnesota (Table 1). These fields had received the maximum or near maximum legal amount of TPTH during a growing season (Robert Skelton, MinnDak, personal communication). The two fields that had tolerant isolates also had leaf spot severe enough to require additional treatment. Leaf spot severity was at least 3%, a level at which economic damage occurs (Smith and Ruppel, 1973; Jones and Windels, 1991). Isolates from these fields were susceptible to thiophanate methyl but tolerant to 5 $\mu g\ ml^{-1}$ mancozeb. Recommended dosage for mancozeb is about 7.5 times higher than that required for TPTH, which accounts for the response to mancozeb.

Table 1. Growth of *Cercospora beticola* isolates from west-central Minnesota on potato-dextrose agar amended with 5 $\mu g\ ml^{-1}$ triphenyltin hydroxide (TPTH), thiophanate methyl (TM) or mancozeb expressed as per cent inhibition of linear growth compared to unamended PDA.

Field	Average percent growth inhibition and the percent of colonies that developed from conidia transferred from 25 leaf spots per field					
	TPTH		TM		Mancozeb	
	%		%		%	
	Inhibition	Colonies	Inhibition	Colonies	Inhibition	Colonies
A	24	84	100	0	5	80
B	100	0	100	0	10	100
C	100	0	100	0	21	80
D	25	92	100	0	6	84
E	100	0	100	0	4	80

Isolates that were tolerant to 1 μg ml^{-1} TPTH were recovered from each of five leaves from a single southern Minnesota field (Table 2); none grew on 5 μg ml^{-1}. Isolates resistant to thiophanate methyl were recovered from three of the five leaves. From one of these leaves (leaf B), no strains were resistant to 1 μg ml^{-1} thiophanate methyl (100% inhibition) but some were resistant to 5 μg ml^{-1} (10% inhibition). This discrepancy probably was because the mass of conidia that were transferred from the leaf spots to 1 μg ml^{-1} thiophanate methyl contained all sensitive strains and those transferred to 5 μg ml^{-1} contained at least one resistant strain. More isolates were tolerant to TPTH than to thiophanate methyl, and the inhibition caused by TPTH was 70 to 79%. Those isolates that were resistant to thiophanate methyl were inhibited only 5 to 10%. The term tolerant should be used in connection with tin fungicides, because the loss of effectiveness of tin is partial, whereas the loss of effectiveness of thiophanate methyl is complete or nearly so. These results support the observation of Giannopolitis (1978) that the loss in effectiveness of organic tin fungicides against *C. beticola* would not be as complete as was the case with benzimidazole fungicides.

Table 2. The effect of triphenyltin hydroxide (TPTH) and thiophanate methyl (TM) on *Cercospora beticola* isolates from a southern Minnesota sugarbeet field expressed as percent inhibition of linear growth when compared to the unamended medium.

Leaf	Fungicide	Percent inhibition of linear growth 1 μg ml^{-1}		5 μg ml^{-1}	
		%		%	
		Inhibition	Colonies	Inhibition	Colonies
A	TPTH	70	91[a]	100	0[a]
	TM	0	13	5	27
B	TPTH	72	90	100	0
	TM	100	0	10	33
C	TPTH	72	47	100	0
	TM	16	47	8	14
D	TPTH	76	100	100	0
	TM	6	33	100	0
E	TPTH	79	100	100	0
	TM	0	87	100	0

[a] The percentage of colonies that grew per number of leaf spots that were transferred.

The response to TPTH of 57 randomly selected isolates in this study are listed in Table 3. At 0.125 $\mu g\ ml^{-1}$ TPTH, over half of the 57 isolates were inhibited only 11 to 30%. At higher concentrations, the inhibition increased. At 0.5 and 1 $\mu g\ ml^{-1}$ TPTH, three and two isolates, respectively, were inhibited 51 to 70%. The level of tolerance among the isolates was variable. About 50% of this population was tolerant to the lowest concentration of TPTH, and 3.5% were tolerant to the highest concentration.

Linear growth of tin-tolerant strains was highly correlated (P = 0.007) to dosages of mancozeb (r^2 = 0.99) but responses to TPTH were not. The growth response was the same at TPTH dosages of 0.250 $\mu g\ ml^{-1}$ and above and also when both fungicides were combined. TPTH was more effective than mancozeb at low rates (Table 4). At the highest rate, mancozeb was as effective as TPTH. No additive effect was apparent when both fungicides were combined. Mancozeb is effective in inhibiting linear growth of the tin-tolerant strains that were used in this test.

Table 3. The effect of four concentrations of triphenyltin hydroxide (TPTH) on the inhibition of linear growth of 57 randomly selected isolates of *Cercospora beticola.*

Percent	TPTH, $\mu g\ ml^{-1}$			
inhibition	0.125	0.250	0.500	1.000
	Number of isolates			
0 - 10	0	0	0	0
11 - 20	12	0	0	0
21 - 30	16	2	0	0
31 - 40	5	1	0	0
41 - 50	2	0	0	0
51 - 60	7	5	2	1
61 - 70	3	18	1	1
71 - 80	1	16	14	9
81 - 90	11	14	28	24
91 -100	0	1	12	22

Table 4. Effect of triphenyltin hydroxide (TPTH) and mancozeb on linear growth of three TPTH-tolerant strains of *Cercospora beticola*.

—— µg ml^{-1} ——		Inhibition of linear growth, %		
TPTH	Mancozeb	TPTH alone	Mancozeb alone	Both fungicides
0.125	0.937	46	18	36
0.250	1.875	82	35	80
0.500	3.750	82	53	82
1.000	7.500	82	87	83

† Mancozeb vs linear growth, $r^2 = 0.99$, $P = 0.007$.

The onset of tolerance can be delayed or eliminated by reducing the exposure time of the fungus to the fungicide (Delp, 1988). Therefore, alternating or tank-mixing TPTH with effective fungicides such as mancozeb should keep tin-tolerant strains to a low and manageable level, especially at the early stage of a buildup of tolerance. If tolerant strains reach an economically important level, then a temporary switch to an alternate fungicide might be effective, because tolerant strains do not survive well and should die-out (Giannopolitis and Chrysalyi-Tokousbalides, 1980). However, the time for the tolerant strains to be eliminated has not been determined under field conditions.

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