Cercospora beticola Tolerant to Triphenyltin Hydroxide and Resistant to Thiophanate Methyl in North Dakota and Minnesota

L. G. Campbell[1], G. A. Smith[1], H. A. Lamey[2] and A. W. Cattanach[3]

[1]USDA-ARS, Northern Crop Science Laboratory, Fargo, ND 58105-5677; [2]Plant Pathology and [3]Soil Science Departments, North Dakota State University, Fargo, ND 58105

ABSTRACT

Triphenyltin hydroxide (TPTH) has been used extensively for control of Cercospora *(Cercospora betiola)* leaf spot of sugarbeet *(Beta vulgaris)* in Minnesota and North Dakota following the development of benzimidazole resistant strains in the early 1980s. The discovery of tolerance to TPTH in 1994 prompted extensive sampling throughout the region in 1995 and 1996. In 1995, 60% of the leaf spots in the southern most district were tolerant to 0.2ppm TPTH and 42% tolerant to 1ppm. By 1996 these frequencies had increased to 83 and 60%, respectively. More alarming than this increase in the southern district was the rapid increase in the occurrence of tolerance further north where the disease is generally less severe and fungicide use is less. In four of the seven factory districts the frequency of leaf spots tolerant to 0.2ppm exceeded 35% and the frequency tolerant to 1ppm was greater than 15%, in 1996. Resistance to thiophanate-methyl, a benzimidazole-type fungicide, persisted in local populations even though TPTH has been the predominant fungicide for control of Cercospora leaf spot for about 15 years.

Additional Key Words: *Beta vulgaris* L., systemic fungicides, protectant fungicides, disease management, leaf spot.

Cercospora leaf spot, caused by the fungus *Cercospora beticola* Sacc., is one of the most widespread and destructive diseases of sugarbeet *(Beta vulgaris* L.). Control measures often combine planting of moderately resistant hybrids with multiple fungicide applications. Many sugarbeet breeders have chosen to concentrate efforts on yield and resistance to other pests at the expense of Cercospora resistance. This is, in part, because resistance is not simply inherited (Bilgen 1969; Smith and Gaskill, 1970; Smith and Ruppel 1974) and in part, because breeders have had limited success in producing highly resistant hybrids with competitive yield potential (Shane and Teng, 1992; Miller et al., 1994; Smith and Campbell, 1996). Continued long-term reliance on fungicides for control of Cercospora leaf spot requires acknowledging that current fungicides may become unavailable because of environmental and health concerns, that development of new fungicides is costly and time consuming , and that *C. beticola* has developed strains with resistance to widely used fungicides.

Benzimidazole fungicides (benomyl, thiabendazole, and thiophanate-methyl) were first used for Cercospora leaf spot control in northern Greece in 1969 and by 1972 Cercospora damage to the foliage of sugarbeet treated with these fungicides was similar to the damage on untreated sugarbeet, under severe disease conditions (Georgopoulos and Dovas, 1973). Benzimidazole resistant *C. beticola* strains and susceptible strains were similar in their abilities to produce the disease on sugarbeet in the absence of fungicides. Triphenyltin fungicides were used prior to the widespread use of the benzimidazoles and after the development of benzimidazole resistant *C. beticola* strains. Inconsistencies in the effectiveness of triphenyltin fungicides in 1976 and 1977 were explained by an increase in the prevalence of triphenyltin tolerant *C. beticola* strains (Giannopolitis, 1978). Differences in growth rate among triphenyltin tolerant *C. beticola* isolates on media containing triphenyltin acetate appeared to be directly proportional to the amount of triphenyltin fungicides used in the area where the isolate originated.

Benzimidazole-resistant *C. beticola* strains were first verified in the USA in 1973 after a few years of intensive utilization of benomyl in Texas (Ruppel and Scott, 1974). Benzimidazole resistance appeared to be responsible for diminished leaf spot control in Arizona in 1974 and 1975 (Ruppel, et al., 1976) and was recognized as a problem in North Dakota and Minnesota in the early 1980s (Percich et al., 1986). Triphenyltin hydroxide became the primary fungicide for leaf spot control fol lowing the development of resistance to the benzimidazole fungicides. Following the discovery of triphenyltin-tolerant *C. beticola* strains in southern Minnesota in 1994 (Bugbee, 1995), the future of leaf spot control in the Northern Plains of the US again became a concern.

This report documents the geographical distribution and increase of fungicide-resistant *C. beticola* in Minnesota and eastern North Dakota after the identification of triphenyltin tolerant strains in 1994. In addition to survey data, results from a fungicide trial provide insight into the impact of some leaf spot management options on the prevalence of tolerant or resistant strains. This information makes apparent the need for and will facilitate the formulation of alternative leaf spot control strategies.

MATERIALS AND METHODS

Leaves were collected from production fields by Agriculturalists from each of the seven factory districts in the region (Renville, MN [Southern Minnesota Beet Sugar Cooperative]; Wahpeton, ND [Minn-Dak Farmers Cooperative]; Moorhead, MN; Hillsboro, ND; Crookston, MN; East Grand Forks, MN; and Drayton, ND [American Crystal Sugar Co.]) in 1995 and 1996. Approximately ten leaves with at least a few visible leaf spots were collected from each field. Conidia of *C. beticola* were transferred from the leaf spots to culture media with the aid of a dissecting microscope and a micropipette. The micropipette contained 3 µl of distilled water with a trace of bromophenol blue to identify inoculation sites on the agar medium. Conidia were dislodged from individual leaf spots with the water and then aliquoted to the culture dishes with the micropipette.

The culture media were prepared by adding technical grade triphenyltin hydroxide (97.1% a.i.) or thiophanate-methyl (95.7% a.i.; dimethyl 4,4'-o-phenylenebis[3-thioallophanate]) that had been dissolved in acetone to autoclaved potato-dextrose-agar (PDA) after the PDA had cooled to 55 C. Lids were kept off the poured culture plates for 30 min to allow dissipation of the acetone in a sterile laminar-flow hood. Streptomycin sulfate (300 ppm) and carbenicillin (50 ppm) were added to the cooled PDA to retard bacterial growth. The culture dishes contained PDA with 0.2 ppm or 1 ppm triphenyltin hydroxide (TPTH) or 5 ppm thiophanate-methyl (TM) (Bugbee, 1995).

The cultures were incubated at 22 C. *Cercospora* colonies of at least 2-mm diameter five days after transfer were considered tolerant (TPTH) or resistant (TM) in both 1995 and 1996. While collecting the 1995 data, colonies just visible to the naked eye after five days were observed on some culture plates containing TPTH. Apparently, some spores had germinated but had grown very slowly. In 1996 these slow growing colonies were counted, added to the number that formed colonies, and reported as an additional variable, percent germination.

C. beticola conidia also were transferred to PDA media that did not contain TPTH or TM to confirm the viability of the spores in the absence of fungicides. On media with no fungicide, 95 to 100% of the leaf spots sampled produced colonies of at least 2-mm diameter within 5 days.

In addition to sampling commercial fields, data were collected from a Cercospora fungicide trial near Breckenridge, Minnesota (Wahpeton factory district) in 1996. This trial compared six registered fungicide treatments and an untreated control. The experimental design was a randomized complete block with four replicates (six replicates for leaf spot damage ratings and extractable sugar yield). Field plots were six rows wide (56 cm row spacing) and 11 m long. The commercial hybrid 'VDH 66156' was planted 23 May on a field than had been in wheat in 1995. Populations of 86,000 plants ha^{-1} were established. Weeds were controlled with herbicides, cultivation, and hoeing. Fungicides were sprayed (30.6 liter ha^{-1} at 8.4 kg cm^{-2} pressure) on the four center rows and data collected from the center two rows of each plot. Seven, 10, and 14-day spray intervals resulted in 6, 4, and 3 fungicide applications, respectively. Leaf spot severity was rated on the 1 (no damage) to 9 (severe damage) KWS scale (Shane and Teng, 1992) on 20 September. A ten-leaf sample from each plot was brought into the laboratory. Assays for tolerance to TPTH and/or resistance to TM were handled as described for the field samples. The trial was harvested with a commercial type harvester on 7 October 1996.

The term tolerance will be used to describe the partial loss of effectiveness of TPTH, in contrast to TM resistance which is characterized by complete, or nearly complete loss of effectiveness (Bugbee, 1995).

RESULTS AND DISCUSSION

C. beticola strains with tolerance to TPTH were first identified in southern Minnesota in 1994 (Bugbee, 1995). One year later TPTH tolerance was found in more than 90% of the fields sampled in the Renville factory district (Table 1). The frequency of fields with TPTH tolerant strains decreased to the north, with a substantial decrease north of Wahpeton in 1995. The distribution of TPTH tolerant strains shifted northward in 1996, with Wahpeton similar to Renville and frequencies at Moorhead similar to occurrences at Wahpeton in 1995. The differences in frequencies between northern and southern districts were less in 1996 than in 1995. The distribution of fields in which TM resistance was found followed a north-south pattern similar to that for TPTH tolerance. Resistance to TM, a benzimidazole-type fungicide, appears to have persisted in local *C. beticola* populations even though TPTH has been the predominant fungicide for control of Cercospora leaf spot for about 15 years.

The frequency of fields in which TPTH tolerant or TM resistant Cercospora strains occur (Table 1) indicates the probability of an individual grower having resistant or tolerant strains in a field. The presence of these strains may be a problem if TPTH or TM are the principal fungicides used to control leaf spot.. A problem with this measurement is the need for large samples when the incidence of Cercospora leaf spot is low and/or resistant or tolerant strains are rare (sampling may follow a poisson distribution). In spite of this deficiency, this measure may be useful in relating the consequences of various long-term Cercospora leaf spot control practices to growers. The percentage of leaf spots forming colonies on media containing fungicide (Table 2) is a more accurate estimate of the frequency of resistant or tolerant strains in the *C. beticola* population than percent of fields in which resistant or tolerant strains are identified.

The frequencies of tolerant or resistant leaf spots (Table 2) and fields in which tolerance or resistance was identified were similar in geographic distribution for both years. The lower frequency for the high rate of TPTH within each factory district suggests that the growth rate (and reproductive potential) of tolerant strains is inversely proportional to TPTH concentration, that tolerant strains lack general fitness, or both (Giannopolitis, 1978; Bugbee, 1995). The persistence of TM resistant strains in the absence of recent widespread TM usage is con

Table 1. Percent of sampled fields in each factory district in which leaf spots with tolerance to triphenyltin hydroxide (TPTH) or resistance to triophanate methyl (TM) were found in 1995 and 1996.

Factory district		1995 Fields sampled	1995 TPTH 0.2ppm	1995 TPTH 1 ppm	1995 TM	1996 Fields sampled	1996 TPTH 0.2ppm	1996 TPTH 1 ppm	1996 TM
		No.	%	%	%	No.	%	%	%
Renville	(South)	52	96	93	72	55	96	96	65
Wahpeton	↑	34	82	68	35	22	95	95	57
Moorhead	400	39	18	15	3	15	80	60	73
Hillsboro	km	24	0	0	0	21	29	19	5
Crookston		22	9	0	0	28	70	37	33
E. Grand Forks	↓	29	7	7	0	26	58	4	4
Drayton	(North)	30	1	0	3	19	42	10	10

Table 2. Percent of leaf spots sampled that germinated or formed colonies on culture media containing triphenyltin hydroxide (TPTH) or thiophanate methyl (TM) in 1995 and 1996.

	1995				1996					
	Colony formation (>2 mm diameter)								Germination	
	Leaf spots	TPTH			Leaf spots	TPTH			TPTH	
Factory district	cultured	0.2ppm	1 ppm	TM	cultured	0.2ppm	1 ppm	TM	0.2ppm	1ppm
	No.	%			No.	%				
Renville	1684	60	42	72	888	83	60	25	86	60
Wahpeton	798	31	13	35	535	55	48	27	55	47
Moorhead	890	2	1	3	443	47	21	28	52	29
Hillsboro	117	0	0	0	258	13	7	1	41	8
Crookston	926	<1	0	0	845	38	16	12	47	18
E. Grand Forks	522	2	1	0	684	8	<1	<1	43	6
Drayton	689	2	0	2	510	10	2	1	37	7

sistent with reports that the development of resistance to benzimidozole-type fungicides did not adversely affect the general fitness of *C. beticola* and the growth rate of resistant strains was independent of fungicide concentration (Georgopoulous and Dovas, 1973; Ruppel et al., 1980). A comparison of the frequency of spores that formed colonies to the frequency that germinated on media containing TPTH (Table 2) may indicate future problems with TPTH tolerance. In the Hillsboro, East Grand Forks, and Drayton factory districts, a substantial number of spores that germinated failed to form colonies. The slow growth of these spores eliminated them as a threat to the 1996 crop; however, they may be an indication of early stages of development of TPTH-tolerance in these areas. In factory districts with relatively high frequencies of spots forming colonies (Renville, Wahpeton, and Moorhead) essentially all the spores that germinated formed colonies.

The relatively high frequencies of TPTH-tolerant or TM-resistant colonies at Crookston in 1996 (Table 2), compared to adjoining factory districts, appears inconsistent with the otherwise north-south distribution pattern of resistance. Sugarbeet production is more intense around Crookston than around Hillsboro and East Grand Forks; consequently, the incidence of disease and fungicide utilization are greater. These conditions enhance development of resistant *C. beticola* strains.

The effect of fungicide treatment on the frequency of TPTH tolerant or TM resistant colonies provides insight into the ability of *C. beticola* to overcome various control strategies and clues to the effectiveness of different management practices in prolonging the usefulness of existing, and possibly future, fungicides. The relatively low frequencies for both TPTH tolerance and TM resistance in the absence of fungicides indicated that the initial inoculum at the Breckenridge site contained only low levels of resistance (Table 3). TM resistance increased only when TM was applied alone or as a component of a tank-mix. TPTH alone or in combination with Maneb (Pro-tex) had no significant effect on the frequency of TM resistant colonies. Mancozeb had no effect upon the frequency of either TPTH tolerant or TM resistant colonies. This is consistent with Bugbee's (1995) observation that mancozeb effectively inhibits the growth of TPTH-tolerant strains. The frequency of TPTH tolerant colonies increased in all treatments that included TPTH. The selection for TPTH tolerance when TPTH was combined with Maneb (Pro-tex) was similar to selection for tolerance with the low rate of TPTH alone.

Table 3. Effects of fungicide treatments on Cercospora leaf spot severity, sugar yield, and frequency of leaf spots tolerant to triphenyltin hydroxide (TPTH) or resistant to thiophanate methyl (TM), Breckenridge, Minnesota, 1996.

Fungicide (rate/application)	Spray interval	Leaf spot severity	Extractable sugar	Leaf spots forming colonies		
				TPTH		
				0.2ppm	1 ppm	TM
	days	(1 to 9)	– kg/ha –		%	
TPTH (263 g/ha)	14	3.6 b*	10209 bc	31 abc	22 a	19 a
TPTH (350 g/ha)	14	3.2 bc	10104 bc	60 cd	54 c	12 a
TPTH (263 g/ha) + TM (420 g/ha)	14	2.9 c	10577 c	52 bcd	38 bc	72 b
TM (560 g/ha)	14	3.7 b	10293 bc	78 d	54 c	69 b
Mancozeb 3.5F (4 liter/ha)	7	3.4 bc	9809 bc	8 a	4 a	2 a
Pro-tex [Maneb + TPTH] (3.5 liter/ha)	10	3.5 b	10102 bc	23 ab	11 ab	2 a
No fungicide applied	—	7.2 a	8666 a	12 a	5 a	4 a

* Means followed by the same letter are not significantly different at P<0.10 (LSD.).

If the competitive ability of *C. beticola* strains is inversely proportional to their level of tolerance, higher application rates might delay the establishment of tolerant strains. This does not appear to be the case in this trial. The high rate of TPTH produced a higher frequency of tolerant colonies than the low rate and was especially effective in selecting colonies tolerant to the high concentration (1 ppm) of TPTH. If high rates are ever to be effective in prolonging the onset of fungicide resistance, use of high rates should be instituted prior to the onset of resistance (Delp, 1988). And then, any delay caused by increase dosage is likely to be followed by the emergence of highly resistant strains of the pathogen (Wolfe and Barret, 1986).

Both treatments that included TM produced relatively high frequencies of TPTH tolerant colonies (Table 3). When TM was applied alone, the frequency of TPTH tolerant colonies, at both the 0.2 and 1ppm TPTH concentrations, was equal to the frequencies observed with the high rate of TPTH alone. This phenomenon, if confirmed, severely restricts the inclusion of TM, and possibly other benzimidazole fungicides, in management schemes that also include TPTH.

Any proposed resistance management program must take into account the ability of alternative schemes to provide adequate leaf spot control for producers. The TPTH + TM tank mix provided the most effective disease control (lowest disease rating and highest sugar yield) at Breckenridge; however, this treatment also produced relatively high frequencies of both TM resistant and TPTH tolerant colonies, making TPTH + TM unacceptable for resistance management. All of the fungicide treatments provided disease control and fungicide treated plots yielded more than non-treated plots. Differences in leaf spot severity and sugar yield among the fungicide treatments were small and frequently not statistically different.

All of the fungicide treatments probably killed most of the *C. beticola* spores in the initial inoculum, based upon the low frequency of fungicide resistant colonies in the absence of fungicides. This likely slowed disease progression and, combined with a late onset, prevented the resistant strains from having a substantial impact on disease severity. Differences among fungicide treatments probably would have been greater if disease development had not been delayed by dry weather. Apparently, resistant strains can increase rapidly, requiring only a few disease cycles beyond initial infection to become predominant. The fre quency of resistant colonies in the absence of fungicides (Table 3) appears to be lower than the frequency of spores in the local population (Wahpeton, 1995 and 1996; Table 2). This would be consistent with observations that fungicide sensitive strains are more competitive than fungicide resistant strains (Ruppel,1975; Ruppel et al., 1980; Giannopolitis and Chrysayi-Tokousralides, 1980). Also, most of the fields (Table 2) likely had received at least one fungicide application prior to sampling.

The rapid increase of resistance in the fungicide trial (Table 3) is consistent with the rapid increase and spread of resistance throughout the region (Table 1). Confirmation of the trends observed in the fungicide trial will be difficult. Future studies must be located where resistant strains have appeared but are infrequent, eliminating the Renville and Wahpeton areas. Leaf spot occurs less frequently and with reduced intensity as one moves north, often rendering results from field trials in northern areas inconclusive.

The scarcity of alternative fungicides makes resistance management difficult. Fungicide resistant strains will likely persist, at least at low levels, for many years even though use of the selective fungicide is discontinued. The results from Breckenridge (Table 3) suggest caution in recommending some tank-mixes. A combination of tank-mixes and rotating fungicides may be useful (Dekker, 1986); however, identifying management schemes that provide adequate leaf spot control while reducing the development of resistance is not easy. New chemicals should be managed in a manner that prolongs their usefulness. This will be difficult considering the few fungicides available and the community effort required. Hybrids with high levels of leaf spot resistance will be utilized only when effective fungicides are not available, in areas where severe leaf spot epidemics are frequent, or when plant breeders are successful in combining leaf spot resistance with competitive yield potential (Miller et al., 1994). If only leaf spot resistant hybrids were planted in an area, the severity of the disease would likely decrease; however, a few individual producers planting resistant hybrids will have little effect.

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