# A SURVEY FOR THE PREVALENCE AND DISTRIBUTION OF *CERCOSPORA BETICOLA* TOLERANT TO TRIPHENYLTIN HYDROXIDE AND RESISTANT TO THIOPHANATE METHYL IN 2001.

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Triphenyltin hydroxide (TPTH) has been used extensively in the Northern Great Plains in recent years for the control of *Cercospora* leaf spot on sugarbeet. Although mancozeb and, to a lesser extent, the benzimidazole fungicides often are implemented in conjunction with TPTH for optimum leaf spot control, TPTH continues to be widely used. Eminent™ (tetraconazole; TCZ) has been used increasingly on sugarbeet in Minnesota and North Dakota in the past few years; testing for tolerance to this fungicide in sugarbeet fields in MN and ND is presented here for the first time.

Testing in our USDA-ARS Fargo laboratory of *Cercospora* that was isolated from leaf spot in the sugarbeet fields in North Dakota and Minnesota for the tolerance or resistance to fungicides first revealed tolerance to TPTH in 1994. The testing program has continued to the present and has in recent years included surveying for tolerance to mancozeb. Testing for baseline tolerance to tetraconazole is also beginning this year due to its increased use in the Red River Valley and southern Minnesota growing regions. As in previous years, fields in the southern Minnesota growing region and in all factory districts from Wahpeton to Drayton in the Red River Valley were surveyed. Samples were tested for resistance to 5 ppm thiophanate methyl (TM; a benzimidazole fungicide) and for tolerance to TPTH and TCZ at two different exposure levels.

## Experimental Procedures

Symptomatic leaves were received from field scouts and were immediately refrigerated to preserve the integrity of the sample. Spores were identified on individual spots on the underside of the leaf using light microscopy. A suspension of the spores was readily obtained using an aqueous solution containing bromophenol blue (0.1%) as a tracking dye.

Technical grade TPTH (97.1% a.i.) or the benzimidazole fungicide thiophanate methyl (TM; 70% a.i.) were prepared according to Bugbee (1995). Suspensions of these compounds were diluted into molten (55°C) potato dextrose agar (PDA) to the final test concentrations in parts per million (ppm) of active ingredient.

Spores resuspended in the tracking dye solution were transferred to each of seven Petri plates containing unamended potato dextrose agar (PDA), PDA + 0.2 ppm TPTH, PDA + 1.0 ppm TPTH, PDA + 2 ppm TCZ, PDA + 10 ppm TCZ, and PDA + 5 ppm TM. Tetraconazole was dissolved in acetone and used to adjust PDA to 2 and 10 ppm levels of fungicide. The cultures were incubated at 22°C and evaluated for growth at 5 to 7 days post-plating.

## Results and Discussion

For the *Cercospora* fungicide testing program of 2001 a total of 827 bagged leaf samples were tested leading to approximately 6,052 individual spots that were tested for fungicide tolerance. This is a significant reduction in the number of samples tested in previous years (~1,000 samples and ~9,000 spots) and was due to the inability to find good leaf spot symptoms in all fields scouted. Procedures used in previous years were used to determine tolerance of *Cercospora beticola* to TPTH at the 0.2 and 1 ppm levels and resistance to Topsin M at the 5 ppm level. Concentrations for the testing of tolerance to TCZ were recently established in our USDA lab. Although testing for tolerance to mancozeb was done in previously, the testing for tolerance to tetraconazole replaced this test due to the increased use of tetraconazole in recent years.

Figures 1 through 3 show the percent of leaf spots that exhibited tolerance to TPTH and resistance to TM at the indicated fungicide levels. Although the leaf spot severity in 2001 was low relative to that in recent years, the proportion of spots harboring *C. beticola* spores that give rise to tolerant isolates remains high, with a minimum of 46% isolates tolerant to 0.2 ppM TPTH in the Crookston factory district (Fig. 1). Fungicide tolerance also remains widely distributed as shown by the high percent of samples that contained at least one leaf spot with tolerance to fungicide (Fig. 2). Resistance to 5 ppm TM also remains high (Fig. 3), with an interesting exception being the SMSC growing region which has discouraged the use of this fungicide in recent years.

The data show inconsistency with the trends in percent tolerance observed in recent years. The percentage of *C. beticola* isolates that exhibited tolerance to TPTH or resistance to TM increased in some grower districts, but decreased in others. Although the result may be due to differential sensitivity to TPTH and TM of *C. beticola* isolates that survived exposure to tetraconazole, the basis of these results nevertheless remains obscure.

Baseline sensitivity testing for tolerance in *C. beticola* to tetraconazole (Eminent™) revealed that some isolates in our USDA collection could withstand a 5 ppm level, but were inhibited in their radial growth by 50% relative to their growth on unamended PDA. Testing of isolates from 2001 fields indicated the presence of a low proportion of isolates (maximally 5.5% in the East Grand Forks production region) that exhibited tolerance to 2 ppm TCZ (Figure 4). No significant levels of tolerance to 10 ppm TCZ were observed in *C. beticola* in 2001.

## Summary

Tolerance to TPTH, TM and TCZ was detected in isolates of *C. beticola* sampled from Minnesota and North Dakota sugarbeet fields in 2001. In contrast with previous years, the trend of higher levels of tolerance to TPTH and TM in southern districts as compared to the northern districts was not as distinct. Even with the incorporation of tetraconazole into the leaf spot control program since 2000, high levels of tolerance to the traditional compounds used for leaf spot control were observed. The greatest increase in fungicide tolerance was recorded in the Red River Valley, whereas the SoMSC growing region experience the least amount of tolerance increase. Late planting, a shorter growing season, and climatic conditions preventative to

Cercospora leaf spot development may have played a role in the decreased severity of *C. beticola* in 2001, but does not appear to have reduced the percentage of fungicide tolerant isolates. Preliminary detection of tolerance to TCZ in 2001 indicates a need for further monitoring of tolerance to this fungicide. Inclusion of testing for resistance/tolerance to TCZ will be done as deemed necessary based on limited small-scale screening of grower samples.

**References:**

Bugbee, W.M. (1995) *Cercospora beticola* tolerant to triphenyltin hydroxide. J. Sugarbeet Res. 32:167-174.