A SURVEY FOR THE PREVALENCE AND DISTRIBUTION OF *CERCOSPORA BETICOLA* TOLERANT TO TRIPHENYLTIN HYDROXIDE AND MANCOZEB AND RESISTANT TO THIOPHANATE METHYL IN 2000.

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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 the most widely used compound for control of the disease. Eminent™ (tetraconazole) has been used on sugarbeet in Minnesota and North Dakota only in the past few years; preliminary testing for tolerance to this fungicide is presented in this year's study.

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 now includes surveying for tolerance to mancozeb. Testing for baseline tolerance to tetraconazole is also beginning this year, as this represents new chemistry available to the grower for the control of leaf spot disease. 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 thiophanate methyl (TM; a benzimidazole fungicide) and for tolerance to TPTH and mancozeb 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.), mancozeb (75% a.i.) or the benzimidazole fungicide thiophanate methyl (TM; 70% a.i.) were prepared according to Bugbee (1995). Mancozeb (75% a.i.) was suspended in water to 2 mg/ml before use. 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 + 5 ppm Mancozeb, PDA + 10 ppm Mancozeb, and PDA + 5 ppm thiophanate methyl. Tetraconazole was dissolved in acetone and used to adjust PDA to 1, 2, 3, 4, and 5 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 2000 a total of 1,231 bagged leaf samples were tested leading to approximately 11,079 individual spots that were tested for fungicide tolerance. 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 mancozeb were established by Bugbee (1995). Since 1997 was the first year that tolerance to mancozeb was incorporated into the study, both the 5 and the 10 ppm levels were used in order to examine what levels of tolerance might exist in the field populations of *C. beticola*. Figures 1 and 2 show the percent of leaf spots that exhibited tolerance to TPTH and resistance to TM at the indicated fungicide levels.

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. The result may be due to differential sensitivity to TPTH and TM of *C. beticola* isolates that survived exposure to tetraconazole, but the basis of these results remains obscure.

Tolerance of field isolates of *C. beticola* to mancozeb was tested for the first time in 1997 and continued into 2000. Tolerance to mancozeb at the 5 ppm level was observed in most districts in 2000, whereas tolerance at the 10 ppm was negligible. 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 (Figure 3).

Finally, the results of leaf sampling in 7 separate fields across 3 sampling dates in 2000 reveal the varying nature of fungicide resistance as a function of time. In Figure 4, percent tolerant leaf spots declined at the time of the middle sampling date, before recovering to levels seen at the first sampling date. The data are consistent with time-of-sampling studies done in 1999 although the basis for the result is unknown. Additional sample date studies will be performed in 2001 to validate the observation.

Summary

Tolerance to TPTH, TM and mancozeb was detected in isolates of *C. beticola* sampled from Minnesota and North Dakota sugarbeet fields in 2000. As in previous years, the trend of higher levels of tolerance to TPTH and TM in southern districts as compared to the northern districts was seen. Even with the incorporation of tetraconazole into the leaf spot control program in 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. Early planting, a long growing season, and climatic conditions conducive to Cercospora leaf spot

development may have played a role in the increase of fungicide tolerance in *C. beticola* populations. Continued detection of tolerance to mancozeb in 2000 indicates a need for further monitoring of tolerance to this fungicide. Inclusion of testing for resistance/tolerance to tetraconazole 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.