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SENSITIVITY OF *CERCOSPORA BETICOLA* TO FOLIAR FUNGICIDES IN 2003.

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Leaf spot, caused by the fungus *Cercospora beticola*, is a constant and consistent disease of sugarbeets produced in the Northern Great Plains area of North Dakota and Minnesota. It causes a reduction in photosynthetic area thereby reducing both yield and sucrose content of the beets. The disease is controlled by crop rotation, resistant varieties and timely fungicide applications. *Cercospora* leaf spot usually appears in the last half of the growing season, and two or three fungicide applications are made during this time for disease control. The most frequently used fungicides are the tin compounds SuperTin and AgriTin (triphenyl tin hydroxide), Topsin (thiophanate methyl), several brands of mancozeb, Eminent (tetraconazole), Gem (trifloxystrobin) and, Headline (pyraclostrobin), which was registered for the first time in 2003. Tin, Topsin and mancozeb are often applied as tank mixes.

Like many other fungi, *C. beticola* has the ability to adapt and become less sensitive to the fungicides used to control them, especially if they are applied frequently over a period of time. The terms sensitive, reduced sensitivity, insensitive, tolerant and resistant are often used to describe the reactions of fungal populations to fungicides. Tin tolerance was first observed in 1994, and has continued at low levels since then. Resistance to Topsin is widespread in *C. beticola* isolates from most beet production areas in the US, including the Northern Great Plains. Reduced sensitivity to mancozeb has not been observed in any fungal pathogen, including *C. beticola*.

We have had an ongoing project evaluating fungicide sensitivity in fungal pathogens since 1992. We have documented reduced sensitivity in several pathogen/fungicide systems of potato, including: *Fusarium sambucinum*: thiabendazole and thiophanate methyl; *Helminthosporium solani*: thiabendazole, thiophanate methyl, and fludioxonil; *Phytophthora infestans*:metalaxyl/mefenoxam; *Pythium ultimum*:metalaxyl/mefenoxam; *Alternaria solani*: chlorothalonil and azoxystrobin, pyraclostrobin, trifloxystrobin, fenamidone, famoxadone. In addition, we have extensive experience monitoring sensitivity of *Cercospora beticola* to fungicides. We began testing *C. beticola* populations for sensitivity to tin in 1996, and continued and expanded sensitivity testing to additional fungicides in subsequent years. From 1997-1999 we conducted tin and Topsin testing. In 2000, we tested 426 *C. beticola* isolates for tin tolerance. In 2001, we utilized our extensive culture collection of *C. beticola* isolates from 1997 on to establish baseline sensitivities to Eminent and Headline, and to evaluate shifts in sensitivity to tin and Topsin that occurred. Multiple isolates from our culture collection were revived from each the years 1997, 1998, 1999 and 2000, and tested for sensitivity to the four most commonly used fungicides in our area: Tin, Topsin, Eminent, and Headline. Fungicide sensitivity was evaluated by radial growth (Tin, Topsin) or spore germination (Eminent, Headline) and an EC50 calculated for each isolate/year. The results indicate a shift toward reduced sensitivity over time to Tin and Topsin, but no change in sensitivity to Eminent or Headline. This test also established a baseline sensitivity of *C. beticola* to Eminent and Headline since some of the isolates tested were collected prior to their use in our sugarbeet region.

In 2002, in cooperation with Dr. John Weiland, Griffin LLC, Sipcam Agro, and Cerexagri, we did extensive sensitivity testing of *C. beticola* isolates collected from throughout the sugarbeet growing region of ND/MN in 2002 to Tin, Topsin and Eminent. Duplicate samples were collected from each field, and one set of leaves delivered to our lab and one set of leaves to Dr. Weiland's lab for sensitivity testing. In contrast to Dr. Weiland, we tested each isolate individually, and calculated an EC50 for each isolate. We tested eight isolates from 107 fields representing all the production areas from southern MN to Drayton. A total of 855 isolates were tested, plus representative isolates from fungicide plots of Dr. Mohamed Khan and Dr. Larry Smith.

The results of our 2002 sensitivity testing were distributed to appropriate industry and research personnel. This information should be useful to the sugar beet industry in order to map areas of reduced sensitivity and monitor changes over time in order to provide area-based fungicide management recommendations.

In 2003, fungicide sensitivity testing was done solely at our facility at NDSU; no additional testing was done by Dr. Weiland. This report presents our test results to date.

OBJECTIVES

The 2003 objectives were:

1) Continue to evaluate sensitivity of *Cercospora beticola* collected from fields representing the sugarbeet production area of the Red River Valley region to SuperTin and AgriTin (triphenyl tin hydroxide), Topsin (thiophanate methyl) and Eminent (tetraconazole).

2) Evaluate sensitivity of *Cercsospora beticola* collected from fields representing the sugarbeet production area of the Red River Valley region to pyraclostrobin fungicide (Headline) and compare sensitivity to our previously established baseline.

3) Distribute results of sensitivity testing in a timely manner in order to make disease management decisions based on test results.

METHODS AND MATERIALS

In 2003, with financial support of the Sugarbeet Research and Extension Board of ND and MN, Griffin LLC, Sipcam Agro, NuFarm, Cerexagri and BASF Corporation, we conducted extensive testing of *C. beticola* isolates for sensitivity to Tin, Topsin, Eminent and Headline collected from throughout the sugarbeet production regions of ND/MN.

Field collection of leaves was done by agronomists and scouts of factory districts as in the past and labeled to include factory district. Leaves were delivered to our lab, and processed immediately to insure viability of spores. From each field sample consisting of 3-5 leaves, *C. beticola* spores were collected from a minimum of five spots/leaf from each leaf of each sample. The spores were mixed, and 200 ul of spores transferred to each of three Petri plates containing water agar amended with Tin at 1 ppm or Topsin at 5 ppm or non-amended (water agar alone). The non-amended media contains ampicillin to prevent bacterial contamination of sub cultures for Eminent and Headline sensitivity testing. Germination of 100 spores viewed at random was done 16 hrs after plating and percent germination calculated.

The fungicide sensitivity testing for Eminent used a standard radial growth procedure developed in our lab for *C. beticola*. A subculture from the original non-amended media was grown on water agar medium amended with ten-fold serial dilutions of Eminent from 0.001 – 1.0 ppm. After 15 days, inhibition of growth was measured, and compared to the growth on non-amended water agar medium. This data was used to calculate an EC50 value for each isolate (EC50 is the concentration of fungicide that reduces growth of *C. beticola* by 50% compared to the growth on non-amended media).

For the strobilurin fungicides, including pyraclostrobin (Headline), the radial growth procedure does not work. Instead, we must use a procedure that measures inhibition of spore germination. We will use a technique developed in our lab by Rivera et al for spore production and sensitivity testing. A subculture from the original non-amended medium was grown on modified V-8 medium and induced to sporulate abundantly. The spores were collected and transferred to water agar amended with ten fold serial dilutions of Headline from 0.001 – 1.0 ppm. Studies in our lab in 2003 demonstrated that *C. beticola* spores reach >80% germination in about 16 hours with some variability depending on isolate. Consequently, germination of 100 spores viewed at random was done 16 hrs after plating and percent germination calculated. An EC50 was calculated for each isolate (EC50 is the concentration of fungicide that inhibits the germination of *C. beticola* by 50% compared to germination on non-amended media).

RESULTS AND DISCUSSION

During 2003, 1031 individual isolates of *C. beticola* representing all production areas and factory districts were tested. This number includes isolates collected from the field fungicide trials of Dr. Mohamed Khan. A few samples that were submitted for testing were not done, because the spores did not germinate despite repeated attempts. We postulate that the fields from which these samples were collected had recently been treated with a fungicide that interfered with spore germination in the lab..

TIN. In 2003, 32 of 1017 isolates (3.1%) had at least one spore that germinated at 1 ppm of Tin. The range of germination was from 1 – 24%. This compares to the results of Weiland who found sporulation values of individual spots between 2 and 28% (est) in 2002 and 8 and 28% (est) in 2001 depending on factory district. The differences are likely due to changes in methodology of assay. Our 2002 results showed that for Tin, 20.7 % of the isolates tested had an EC50 value of >1 ppm when tested by the radial growth procedure, with an average EC50 of 1.01 ppm. This compares to EC50 values of representative isolates from our collection of previous years of 1.46 (1997), 0.91 (1998), 0.80 (1999) and 0.98 (2000).

TOPSIN. In 2003, 725 of 1017 isolates (71.3%) had at least one spore that germinated at 5 ppm of Topsin. The range of germination was from 1-100%. Our results were higher than the results that Weiland found of 20-40% in 2002 and 20-50%in 2001 (est values) depending on factory district. Our 2002 results showed that for Topsin, 31.8 % of the isolates tested had an EC50 value of >50 when tested by the radial growth procedure, with an average EC50 value of isolates <50 ppm of 3.98. These isolates were either highly resistant, EC50> 50 ppm, or sensitive, EC50 <5 ppm; there were very few intermediate isolates found. This compares to EC50 values of representative isolates from our collection of previous years of 0.23 (1997), 0.06 (1998), 0.04 (1999) and 0.038 (2000). It appears that the incidence of insensitivity to Topsin is increasing.

In 2002, 7.5 % of the isolates had reduced sensitivity to both Tin and Topsin. In 2003, 21 of 954 (2.2 %) had reduced sensitivity to both Tin and Topsin.

However the 2003 results for Tin and Topsin cannot be compared directly to 2002 results since different procedures were use din each year. In 2002, radial growth was used in order to calculate an EC50 value, and in 2003, a spore germination procedure was used with no EC50 value calculated. However, because both Tin tolerance and Topsin resistance have been documented for many years without much change in EC50 values, incidence of tolerance and resistance rather than changes in EC50 are most important. Hence, the spore germination procedure was implemented in 2003.

EMINENT. In 2003, 973 isolates were tested for sensitivity to Eminent, with an average EC50 value of 0.120 ppm.. In 2003, 0.2% of the isolates had EC50 values of <0.001, 6.5% had EC50 values between 0.0011-0.01, 70.3% had EC50 values between 0.011-0.1, 17.1% had EC50 values between 0.11-1.0 and 6.0% had EC50 values >1.0 ppm (Figure 1). Our 2002 results showed that for Eminent, 1.2 % of the isolates tested had an EC50 value of >1 ppm when tested by the radial growth procedure, with an average EC50 value of 0.206 ppm. This compares to average EC50 values of representative isolates from our collection of previous years of 0.128 (1997), 0.093 (1998), 0.115 (1999), and 0.100 (2000) (Figures 2 and 3). It appears that Eminent sensitivity if relatively unchanged since 1997.

HEADLINE. The year 2003 was the first year that Headline sensitivity monitoring was done, since Headline was first registered for the 2003 crop year. However we had established a limited baseline for isolates not exposed to Headline using our collection of *C. beticola* isolates collected in previous years. To date for 2003, of 422 isolates tested, the spore germination rate at 0 ppm pyraclostrobin (the active ingredient of Headline) is 89.8 % with a range of 11-100% germination. The EC50 values for spore germination on Headline amended media were 16.4% at < 0.001 ppm, 53.3 % between 0.0011-0.01 ppm, 27.7% between 0.011-0.10, 2.1% between 0.11 -1.0 and 0.5% at >1.00 ppm (Figure 4).

2003 was a transition year in testing from bulk spore germination done previously by Dr. Weiland, to a combination of percent spore germination at base levels for Tin and Topsin, and EC50 value calculations using radial growth for Eminent and spore germination for Headline. It is more important to monitor changes in sensitivity to Eminent and Headline than incidence, because these fungicides are new and considered the primary fungicides by growers, monitoring of EC50 values is necessary in order to detect small changes in sensitivity of the *C. beticola* poplulations as we have seen in 2003. Because Tin and Topsin tolerance and resistance are well documented and established, it is necessary only to monitor changes in incidence rather that shifts in the population. The procedures used in 2003 will be used in future years so direct comparisons can be made in fungicide sensitivity of *C. beticola* over time in order to adjust fungicide recommendations if necessary and provide resistance management data for registration of new fungicides.

Fungicide sensitivity monitoring is also an important issue in potatoes, particularly for the strobilurin (QOI) fungicides, of which there are four products registered for potatoes: Quadris, Headline, Gem, and Tanos. Decreased sensitivity to Quadris has been documented in the early blight pathogen, *Alternaria solani* after only two years of use. Because *C. beticola* has a history of developing tolerance or insensitivity to fungicides, and insensitivity to at least one, and probably other, strobilurin fungicides has developed in another adaptable potato pathogen, it is important to monitor population sensitivity to Headline. It is also important to monitor sensitivity to Eminent, since this is the alternating fungicide partner for managing reduced sensitivity in Headline.

Figure 1. Sensitivity of 973 *Cercospora beticola* isolates from ND/MN to tetraconazole (Eminent)

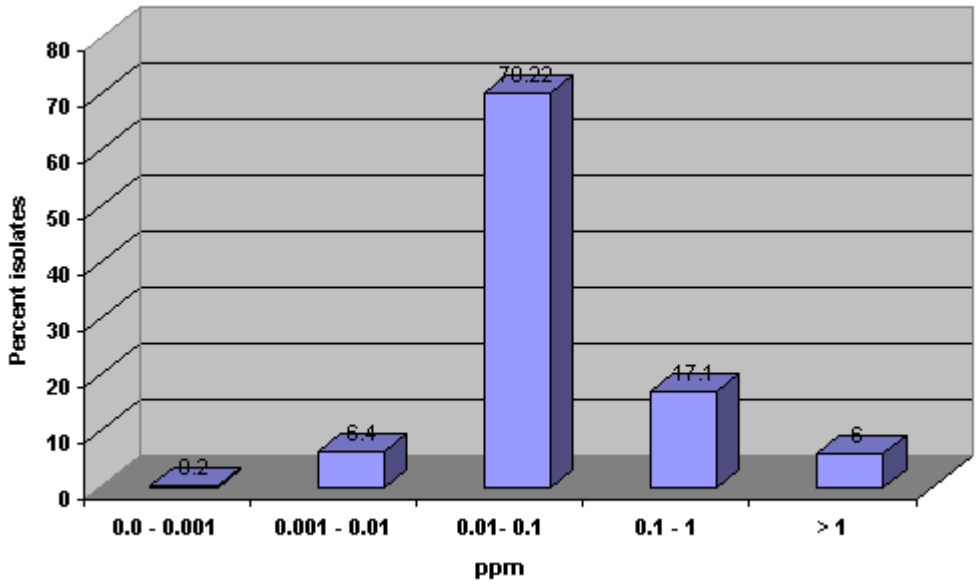


Figure 2. Sensitivity range of *Cercospora beticola* isolates from ND/MN to tetraconazole (Eminent) 1997-2003 as measured by EC-50 values (ppm)

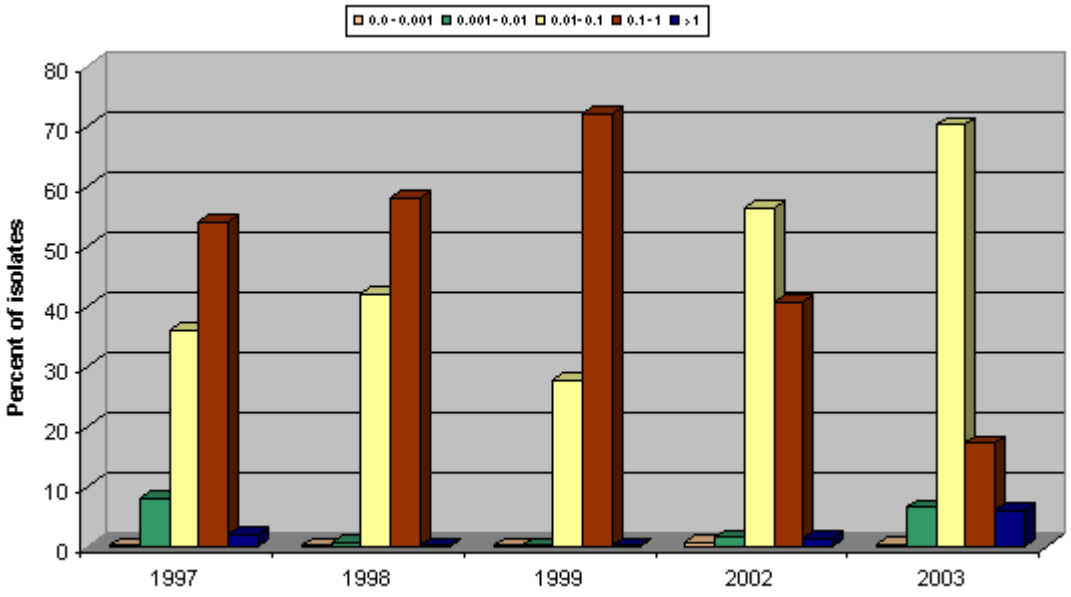


Figure 3. Sensitivity range of *Cercospora beticola* isolates from ND/MN isolates to tetraconazole (Eminent) 1997-2003 measured by EC-50 valyes (ppm)

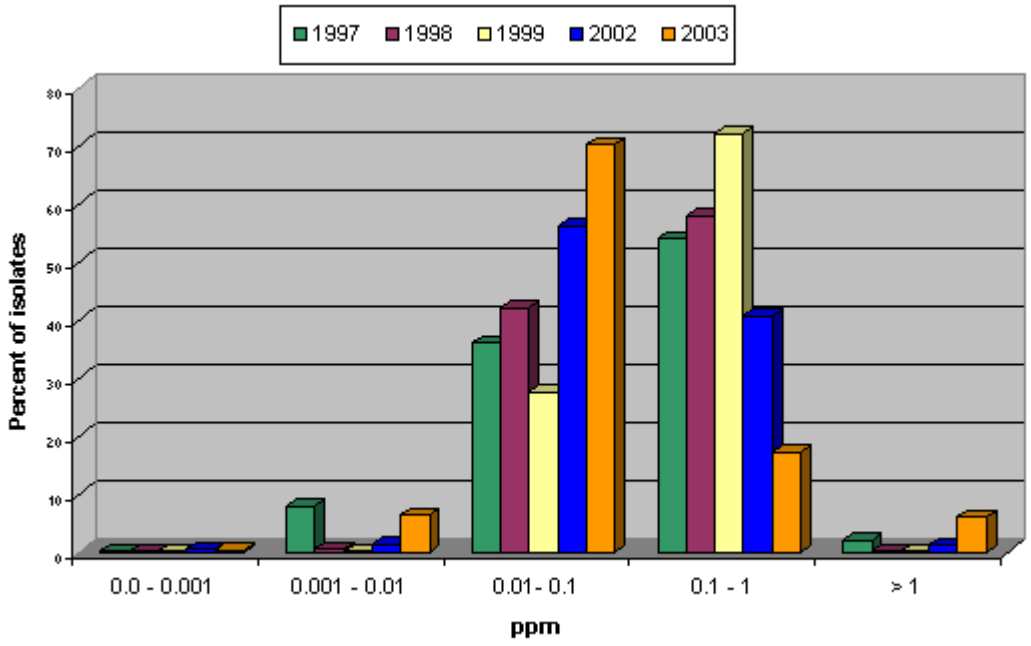
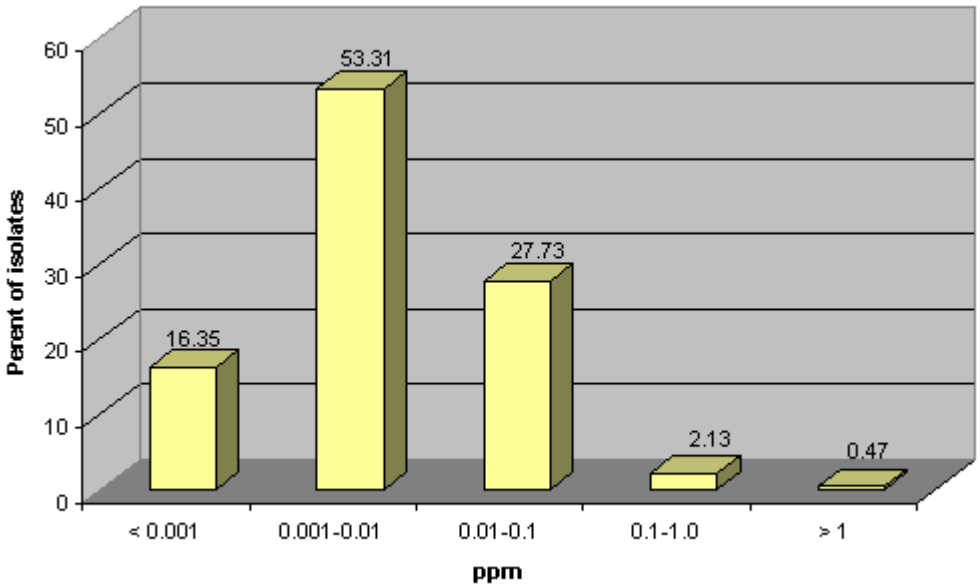


Figure 4. Sensitivity of 422 *Cercospora beticola* isolates from ND/MN to pyraclostrobin (Headline) in 2003


60
50
40
30
20
10
0
Perent of isolates
53.31
27.73
16.35
2.13
0.47
< 0.001
0.001-0.01
0.01-0.1
0.1-1.0
> 1
ppm