SENSITIVITY OF *CERCOSPORA BETICOLA* TO FOLIAR FUNGICIDES IN 2004.

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Leaf spot, caused by the fungus *Cercospora beticola*, is an endemic 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 to four 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), Eminent (tetraconazole), Gem (trifloxystrobin) and, Headline (pyraclostrobin). Tin and Topsin 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

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 been 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 1997-2000 we evaluated sensitivity to tin and thiophanate methyl. We utilized our extensive culture collection of *C. beticola* isolates from 1997-2000 to establish baseline sensitivities to Eminent, Headline and Gem and to evaluate shifts in sensitivity to tin and Topsin.

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 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. In 2003 and 2004 we

continued fungicide sensitivity testing of *C. beticola* to the commonly used fungicides in our area. Testing was conducted only at NDSU.

OBJECTIVES

The 2004 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 (Headline) and trifloxystrobin (Gem) fungicides and compare sensitivity to previously established baselines.

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 2004, with financial support of the Sugarbeet Research and Extension Board of ND and MN, DuPont, Sipcam Agro, Cerexagri, BASF Corporation and Bayer Crop Science, we conducted extensive testing of *C. beticola* isolates collected from throughout the sugarbeet production regions of ND/MN for sensitivity to Tin, Topsin, Eminent, Headline and Gem

Field collection of leaves was done by agronomists and scouts from all factory districts. 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 composite of 200 µl 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 subsequent Eminent, Headline and Gem 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 serial ten-fold 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 EC_{50} value for each isolate (EC_{50} 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 Headline and Gem, the radial growth procedure does not work. Instead, we must use a procedure that measures inhibition of spore germination developed in our lab by Rivera et al for efficient 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 serial ten fold dilutions of Headline or Gem 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 EC_{50} was calculated for each isolate (EC_{50} is the concentration of fungicide that inhibits the germination of *C. beticola* by 50% compared to germination on non-amended media).

RESULTS AND DISCUSSION

Cercospora disease was not prevalent during 2004 due to the abnormally cool season. However, due to the diligent collection efforts of the grower cooperative agronomists, approximately 947 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, Dr. Larry Smith and SMSBC Renville. 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, or that the lesions may have been bacterial leaf spot and not Cercospora leaf spot.

Tolerance to triphenyl tin hydroxide was first observed in 1994, with tolerance levels between 1-2 ppm. The incidence of tin tolerance increased between 1997 and 1999, but incidence of isolates tolerant to triphenyltin hydroxide at 1.0 ppm has been declining since the introduction of tetraconazole for resistance management. In 1998, the percentage of isolates with tolerance to triphenyltin hydroxide at 1.0 ppm was 64.6%, in 1999 it was 54.3%, in 2001 was 34.6%, in 2002 was 20.7%, in 2003 was 3.1% and in 2004 was 1.1% (Fig. 1a). The factory district with the highest incidence of tin tolerance is SMBSC (Fig. 1b), but the number of samples tested was low (15).

Resistance to benzimidazole fungicides became widespread in *C. beticola* in the 1980's in many sugar beet production areas of the US, including the Northern Great Plains. In 1998, 70.8% of the samples were resistant to thiophanate methyl at >5.0 ppm when tested using a bulk spore germination procedure; in 1999, 71.3% of the samples were resistant; in 2001, 56.4% of the samples were resistant; in 2003, 71.3% of the samples were resistant; and in 2004, 78.6% of the isolates were resistant (Fig 2a). It appears that resistance to thiophanate methyl continues to be widespread in the sugarbeet production area of North Dakota and Minnesota and equally distributed among all factory districts (Fig 2b). Consequently, the use of thiophanate methyl is only recommended as a tank mix partner with triphenyltin hydroxide.

In 2002, 7.5 % of the isolates had reduced sensitivity to both triphenyltin hydroxide and thiophanate methyl; in 2003, that figure was 2.2 % of the isolates, and in 2004 it was 0.9% of the isolates.

A baseline sensitivity curve was developed for tetraconazole using *C. beticola* isolates from 1997-1999 that had not been previously exposed to tetraconazole and the year 2000 from our culture collection. The average EC_{50} values of these *C. beticola* isolates is 0.128 (1997), 0.093 (1998), 0.115 (1999), and 0.100 (2000) using a radial growth procedure. The average EC_{50} value of field-collected isolates from 2002 was 0.206 ppm, from 2003 was 0.120 ppm and from 2004 was 0.243 (Fig 3a). In 2002, 1.2 % of the isolates tested had an EC_{50} value of >1 compared to 6.0% of the isolates in 2003 and 10.8% of the isolates in 2004 (Fig 3b). It should be noted that 63.4% of the isolates tested in 2004 had an EC_{50} value of 0.1 ppm. Sensitivity to tetraconazole appears to be similar across factory districts, but the SMBSC district has a higher incidence of isolates with an EC_{50} value between 0.1 and 1.0 compared to other districts (Fig 3b). While it appears that the average tetraconazole sensitivity has remained relatively unchanged since 1997, one concern is with the observed thousand–fold difference among the isolates from most sensitive to least sensitive (Fig. 4) and a slight increase in the incidence of isolates with EC_{50} values >1 ppm over the past three years (Fig 4), perhaps suggesting that there are *C. beticola* isolates in nature with reduced sensitivity to this fungicide class.

No reduction in sensitivity has been documented since the registration of QoI fungicides on sugarbeet, however, a limited baseline has been established using *C. beticola* isolates from our culture collection not previously exposed to pyraclostrobin and trifloxystrobin. Substantial variability exists among the isolates tested, with a thousand-fold difference in EC_{50} values among the isolates to pyraclostrobin in 2003 and trifloxystrobin in 2004 (6a), indicating a potential for reduced sensitivity. It should be noted that we have found isolates in the population that have an EC_{50} value >1.0 ppm for Headline and Gem. Sensitivity among factory districts appears to be the same for these two fungicides (Figs 5b and 6b).

Fungicide sensitivity monitoring is not only important for control of sugarbeet diseases, but is also an important issue in potatoes, particularly for the strobilurin (QoI) fungicides. There are five QoI products registered for potatoes: Quadris, Headline, Gem, Reason and Tanos. Decreased sensitivity to Quadris and Headline 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 and Gem. It is also important to monitor sensitivity to Eminent, since this is the alternating fungicide partner for managing reduced sensitivity in Headline and Gem.

Work is in progress to develop molecular based real-time PCR tests to monitor changes in fungicide sensitivity, particularly for the QoI fungicides. Preliminary primers from *C. beticola* have been developed and analyzed for the presence of nucleotidebase changes.

SUMMARY

1. Tin tolerance at 1.0 ppm is declining, probably due to the use of alternate fungicides that reduce the number of tin applications.

2. Resistance to Topsin at 5.0 ppm is widespread across all production areas of the state, and is not declining.

3. Sensitivity to Eminent is relatively stable, but there is a slow increase in the number of isolates with an EC_{50} > 1.0 ppm which may indicate the potential for reduced sensitivity to develop.

4. There is no increase in the number of isolates with reduced sensitivity to Headline and Gem, but there are rare isolates identified with a thousand-fold decrease in sensitivity.

5. A combination of alternation and combinations of fungicides with different modes of actions may be necessary to prevent reduced sensitivity of *C. beticola* to currently registered fungicides.

6. Work is in progress to develop a molecular based real time PCR for reduced sensitivity screening.

Fig 1a. Sensitivity to TPTH of *C. beticola* isolates collected in ND and MN in 2004 at 1.0 ppm as measured by bulk spore germination

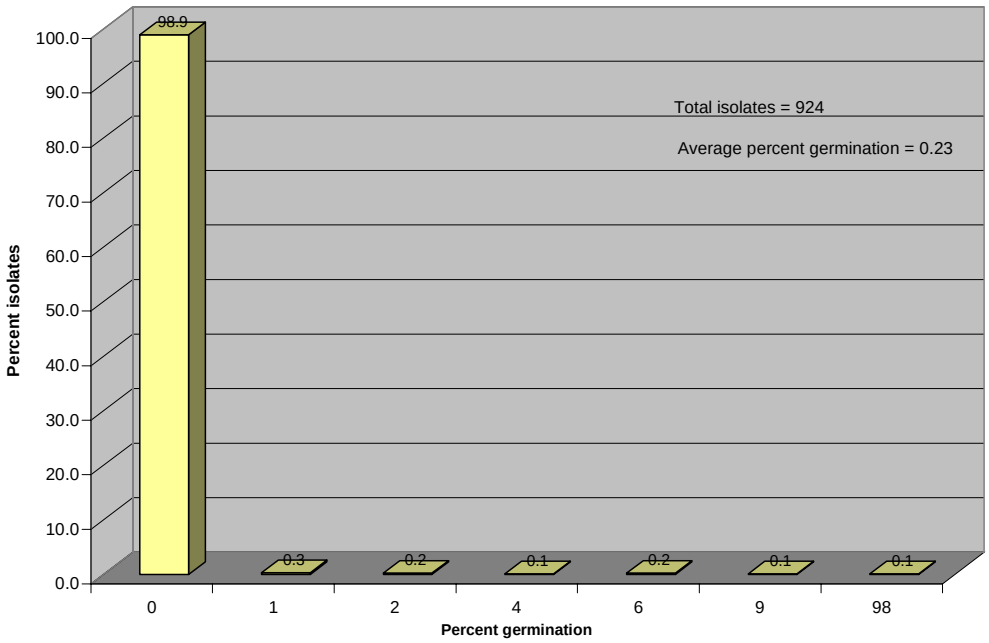


Fig 1b. Tolerance of *C. beticola* to TPTH at 1.0 ppm by factory district 2004

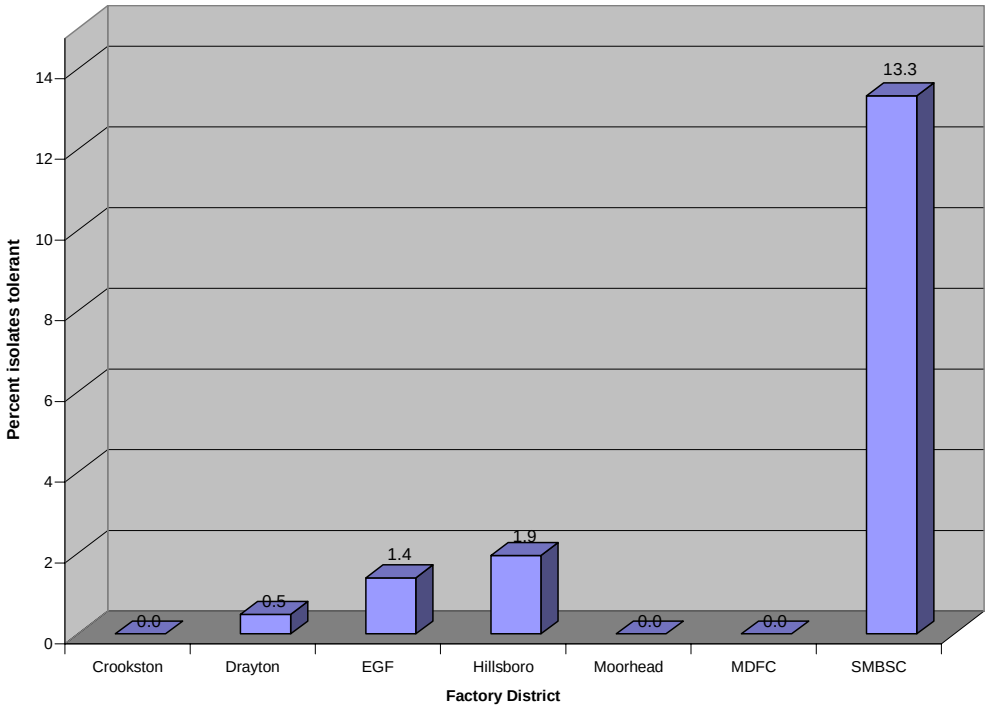


Fig 2a. Sensitivity to thiophanate methyl of *C. beticola* isolates collected in ND and MN in 2004 at 5.0 ppm as measured by bulk spore germination

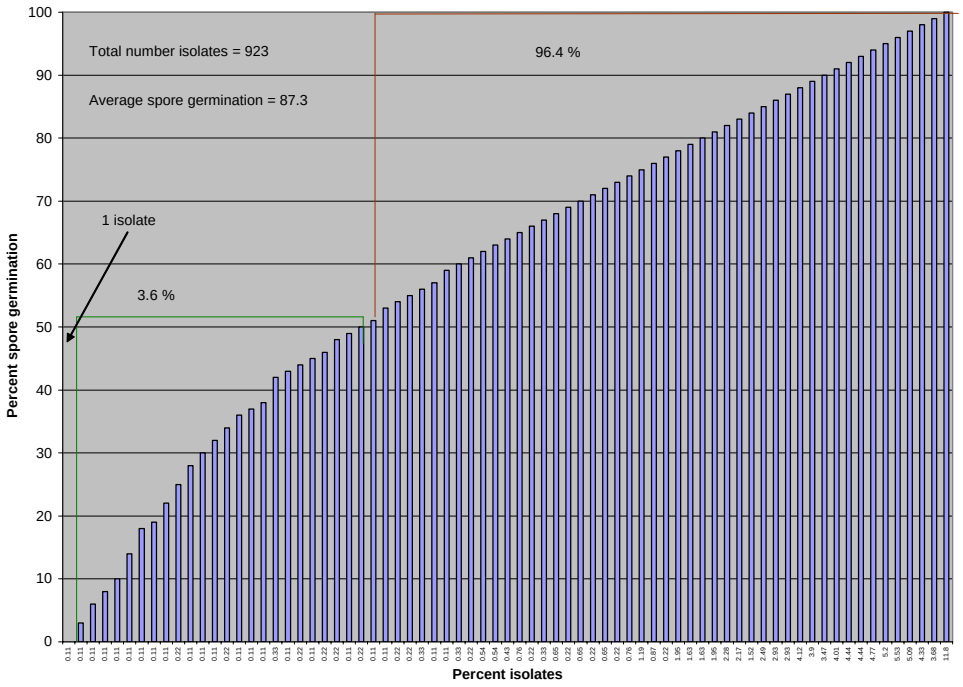


Fig 2b. Resistance of *C. beticola* to thiophanate methyl at 5.0 ppm by factory district 2004

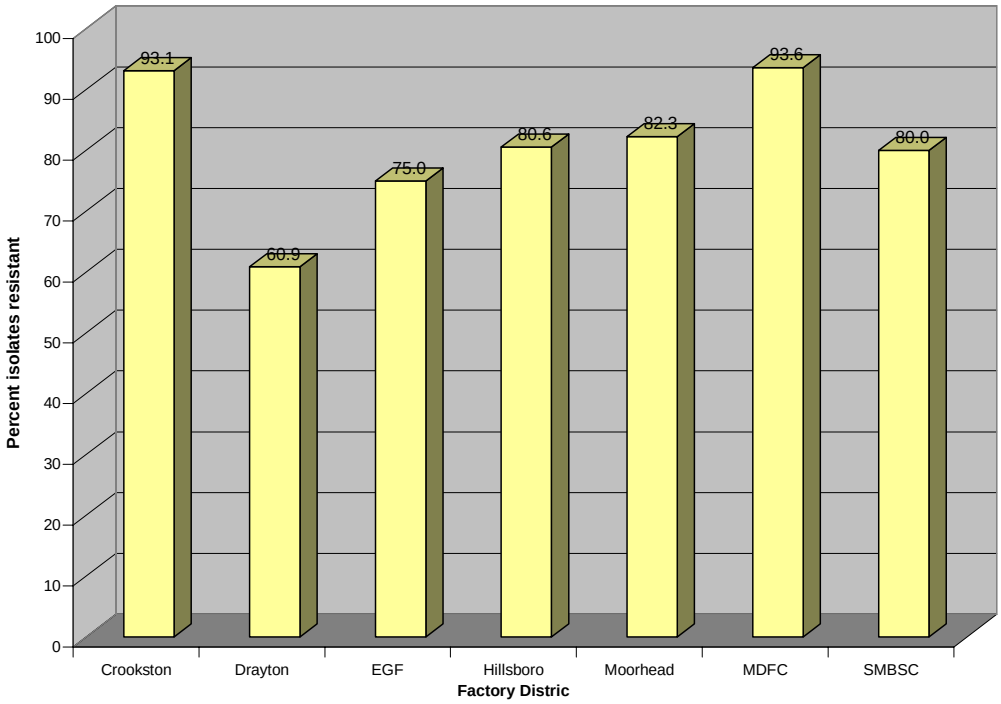


Fig 3a. Sensitivity to tetraconazole of *C. beticola* isolates collected in ND and MN in 2004 as measured as radial growth (EC-50)

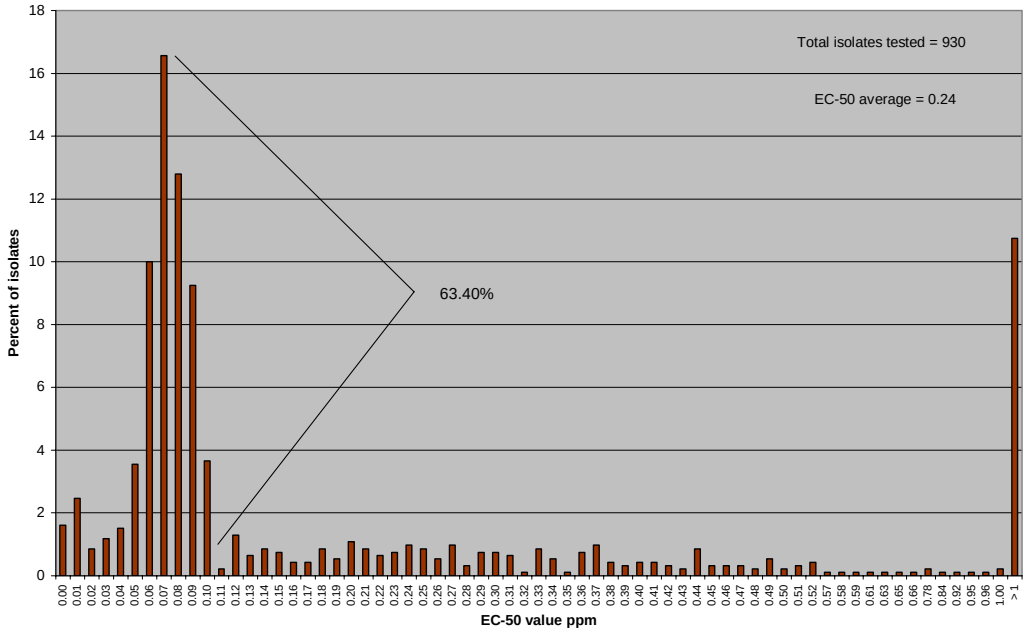


Fig 3b. Sensitivity of *C. beticola* to tetraconazole by factory district 2004

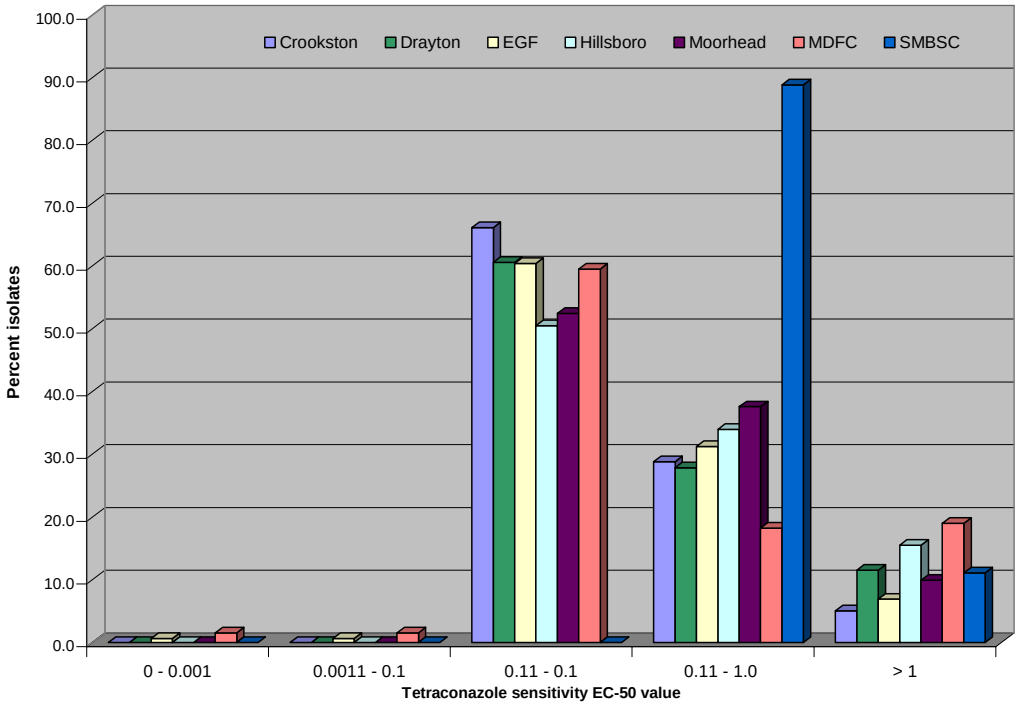


Fig 4. Sensitivity of *C. beticola* isolates collected in ND and MN from 1997-2004 to tetraconazole

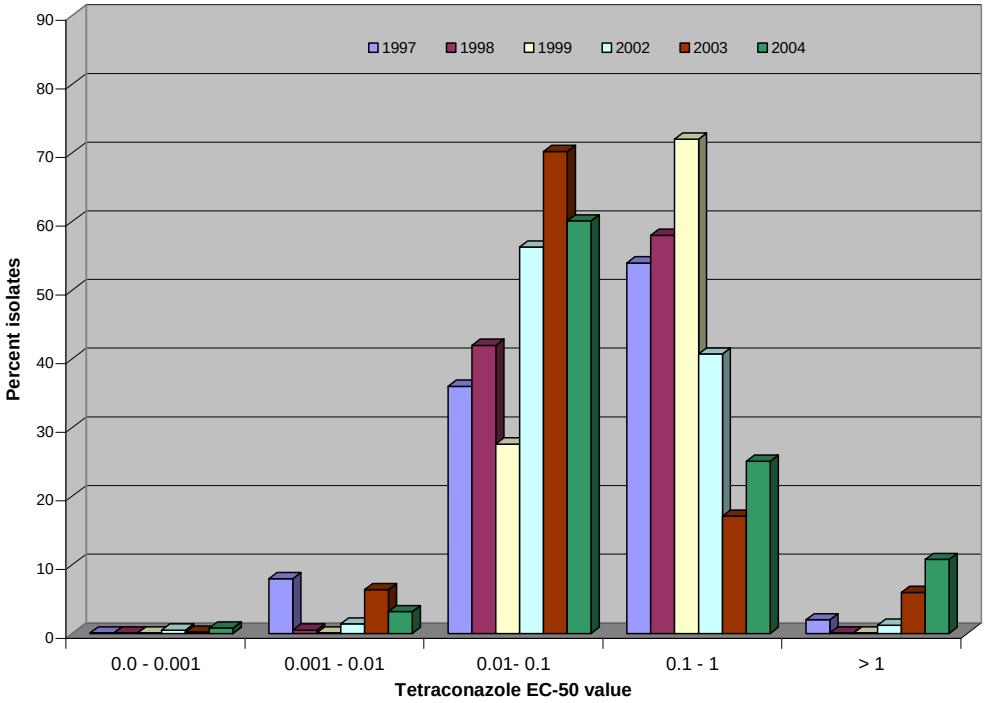


Fig 5a. Sensitivity to pyraclostrobin of *C. beticola* isolates collected in ND and MN in 2004 as measured by spore germination (EC-50)

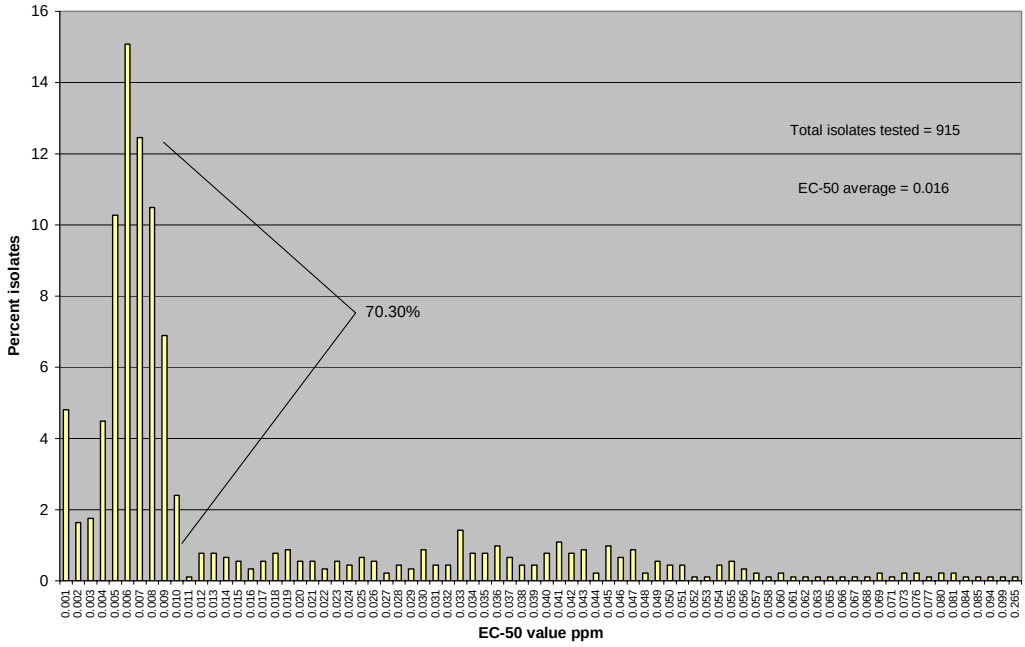


Fig 5b. Sensitivity of *C. beticola* to pyraclostrobin by factory district 2004

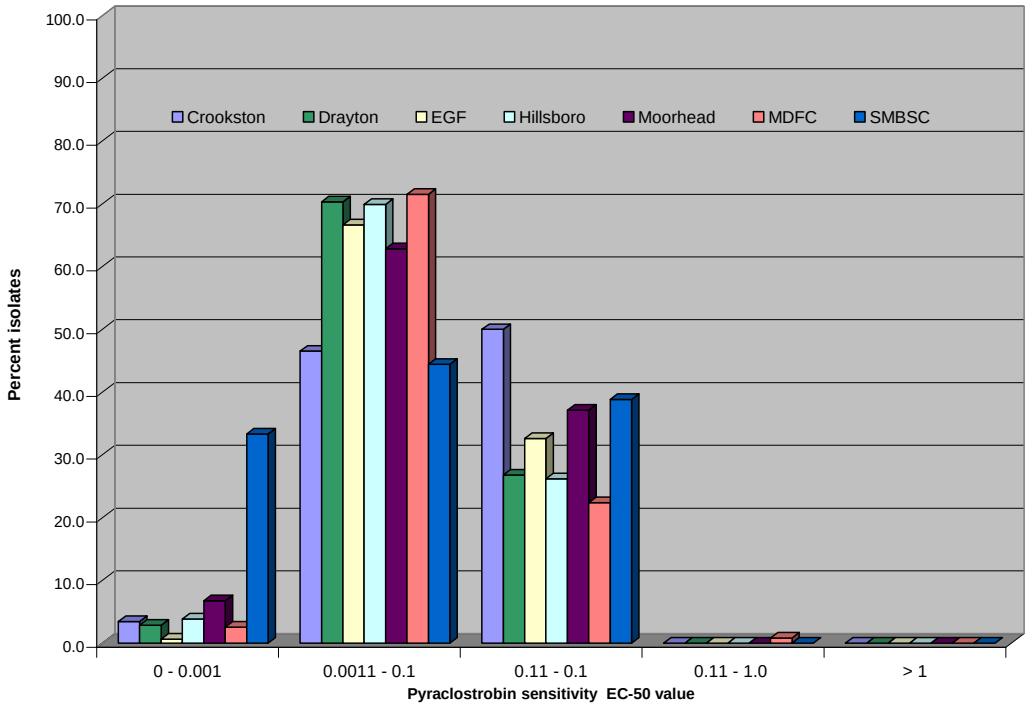
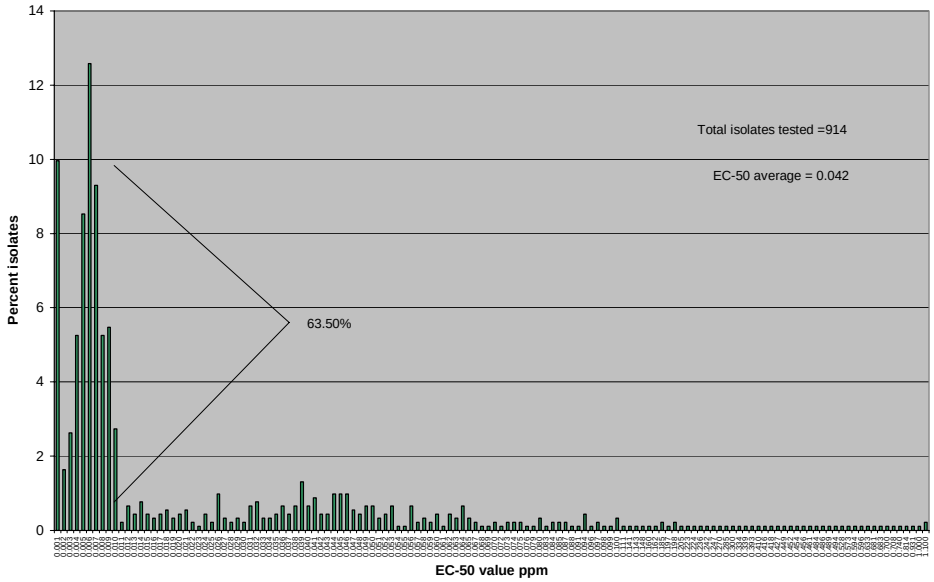


Fig 6a. Sensitivity to trifloxystrobin of *C. beticola* isolates collected in ND and MN in 2004 as measured by spore germination (EC-50)



6b. Sensitivity of *C. beticol*a isolates to trifloxystrobin by factory district 2004