

SENSITIVITY OF *CERCOSPORA BETICOLA* TO FOLIAR FUNGICIDES IN 2006.

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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. Fungicides are alternated and 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 together as a tank mix.

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. 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. Fungicide sensitivity testing of field isolates of *C. beticola* to the five commonly used fungicides in our area has been conducted in the years 2003 - 2006.

OBJECTIVES

The 2006 objectives were:

- 1) Continue to evaluate sensitivity of *Cercospora beticola* isolates collected from fields representing the sugarbeet production area of the Red River Valley region to Supertin (triphenyl tin hydroxide) and Eminent (tetraconazole).
- 2) Evaluate sensitivity of *Cercospora beticola* isolates 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 2006, with financial support of the Sugarbeet Research and Extension Board of ND and MN, DuPont, Sipcam Agro, 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, Eminent, Headline and Gem. Due to the widespread resistance to Topsin, sensitivity testing to Topsin will only be conducted every three years; testing was not done in 2006.

Sugar beet leaves with *Cercospora* leaf spot (CLS) were collected from commercial fields by agronomists from all factory districts. Leaves were delivered to our lab, and processed immediately to insure viability of spores. From each field sample *C. beticola*, spores were collected from a minimum of five spots/leaf from five leaves. The spores were mixed, and composite of 200 μ l of spores transferred to each of two Petri plates containing water agar amended with Tin at 1 ppm or non-amended (water agar alone).

For tin sensitivity, a bulk spore germination procedure was used. Germination of 100 random spores on the tin amended water agar was counted 16 hrs after plating and percent germination calculated.

Germination on non-amended media was calculated and this plate was used as a source of single spore sub cultures for subsequent Eminent, Headline and Gem sensitivity testing.

For tetraconazole fungicide sensitivity testing, a standard radial growth procedure developed in our lab for *C. beticola* was used. A single spore subculture from the original non-amended media was grown on water agar medium amended with serial ten-fold dilutions of technical grade tetraconazole from 0.001 – 1.0 ppm. After 15 days, inhibition of radial 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 Headline and Gem, the radial growth procedure does not work. Instead, we must use a procedure that measures inhibition of spore germination.. A subculture from the original non-amended medium was grown on modified V-8 medium and induced to sporulate abundantly using a procedure developed in our lab for efficient spore production and sensitivity testing The spores were collected and transferred to water agar amended with serial ten fold dilutions of technical grade pyraclostrobin or trifloxystrobin from 0.001 – 1.0 ppm. Previous studies 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). Fresh preparations of Gem (used the day as prepared) were used throughout the study, as some loss of potency with time has been observed in previous testing

RESULTS AND DISCUSSION

Cercospora disease developed late in the 2006 season and the majority of the CLS samples were delivered to our lab in September; 16% of the samples were delivered in August, 76% delivered in September and 8% delivered in October. Due to the diligent collection efforts of the grower cooperative agronomists, 988 field samples were received for testing representing all production areas and factory districts were received and tested. An additional 364 samples were received from fungicide trial plots of Dr. Mohamed Khan (Foxhome), Dr. Larry Smith (Crookston) and Mark Bredehoeft (Renville), and tested for fungicide sensitivity. For this report, only results from the field samples are included; the fungicide trial results are not included. Some samples that were submitted for testing were not done, because the spores did not germinate despite repeated attempts. Of the 988 samples received, 956 samples (97%) were tested. 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 reported 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 triphenyl tin hydroxide at 1.0 ppm has been declining since the introduction of tetraconazole for resistance management in 1999, trifloxystrobin in 2002 and pyraclostrobin in 2003. In 1998, the percentage of isolates with tolerance to triphenyl tin hydroxide at 1.0 ppm was 64.6%, in 1999 it was 54.3%, in 2000 it was 17.7%, in 2001 was 14.9%, in 2002 was 9.0%, in 2003 was 1.1%, in 2004 was 1.1%, in 2005 was 0.97% and in 2006 was 0.0% (Fig. 1). None of the isolates tested in 2006 showed resistance to tin fungicide. The decline in tin tolerance is associated with the use of additional fungicides with different chemistry which resulted in a reduction of average number of tin applications from 2.4 in 1998 to less than one since 2001 (Fig. 1). The average number of tin applications in 2006 was 0.56 (Fig. 1).

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. There appears to be a slow increase in the average EC₅₀ value of CLS isolates from 1998 to 2005 (Fig. 2). The average EC₅₀ values of these *C. beticola* isolates from our culture collection are 0.13 (1997), 0.09 (1998), 0.12 (1999), and 0.23 (2000) using a radial growth procedure. The average EC₅₀ value of field-

collected isolates from 2002 was 0.21 ppm, from 2003 was 0.12 ppm, from 2004 was 0.24, from 2005 was 0.29 and from 2006 was 0.14. These values include isolates with an EC₅₀ value of >1.0 ppm.

In 2002, 1.2 % of the isolates tested had an EC₅₀ value of >1 to tetraconazole compared to 6.0% of the isolates in 2003, 10.8% of the isolates in 2004, 12.4% in 2005, and in 2006 was 7.3% (Fig 3). The trend from 2003 - 2005 has been for increased resistance to tetraconazole as indicated by an increase in both average EC₅₀ values and the incidence of isolates with EC₅₀ values >1 ppm. This is the first indication of a decrease in resistance to tetraconazole, and along with the reduction in tin resistance, may indicate that our collective resistance management program and recommendations are working. Sensitivity to tetraconazole in 2006 appears to be similar across factory districts, but the average EC₅₀ value was highest in the SMBSC district, but SMBSC had no isolates with an EC₅₀ > 1.0 (Figs. 4 and 5).

Baseline sensitivity to the QOI fungicides Headline and Gem was done using *C. beticola* isolates from our culture collection not previously exposed to pyraclostrobin and trifloxystrobin and will be used to monitor shifts in sensitivity to these fungicides. Sensitivity of *C. beticola* to both of these fungicides has remained relatively stable (Figs. 6 and 7) since these fungicides have been used commercially (Headline four years, Gem three years). There is a slight shift toward resistance compared to the baseline with both strobilurin fungicides (Fig. 8), but the shift is less than 10X and may be attributed to natural variation or experimental noise. However, substantial variability exists among the isolates tested, with a thousand-fold difference in EC₅₀ values among the isolates to pyraclostrobin and trifloxystrobin, indicating the potential for reduced sensitivity is present in the population. It should be emphasized that we have found isolates in the population that have an EC₅₀ value >1.0 ppm for both Headline and Gem. It is important to know that there are numerous examples in many crops where resistance has developed to strobilurin (QOI) fungicides due to overapplication and misapplication of these fungicides. Because Gem and Headline are strobilurin/QOI fungicides, it is important to continue to monitor sensitivity of *C. beticola* to these two fungicides.

Because *C. beticola* has a history of developing resistance to fungicides, and has a high degree of variability in cultures, the potential for resistance development to fungicides is always there. We must continue to monitor *C. beticola* populations in our area for fungicide sensitivity/resistance and develop disease management strategies with this goal as a priority.

SUMMARY

1. Tin tolerance at 1.0 ppm has basically disappeared in our region, probably due to the use of alternate fungicides that has resulted in the reduction in the number of tin applications from 2.14 in 1998 to less than one each year since 2001.
2. Resistance to Topsin at 5.0 ppm is widespread across all production areas of the state, and is not declining. Topsin was not tested in 2006.
3. Sensitivity to Eminent is relatively stable, but there has been a slow increase in the number of isolates with an EC₅₀ > 1.0 ppm which may indicate the potential for reduced sensitivity to develop. In 2006 for the first time since testing began, there was a decrease in both the number of isolates with an EC₅₀ value >1.0 ppm and the overall EC₅₀ value across all isolates tested.
4. Sensitivity to Headline and Gem remains relatively stable, but there are rare isolates identified with a thousand-fold decrease in sensitivity. There has been a slight change in sensitivity to Gem and Headline compared to the baseline since use and testing of these compounds began three and four years ago respectively. This change is not a cause for concern.
5. It appears that the fungicide resistance management plan that we are following is working.
6. A combination of alternation and combinations of fungicides with different modes of actions will continue to be necessary to prevent reduced sensitivity of *C. beticola* to currently registered fungicides.

7. Continue to use disease control recommendations currently in place including:

- Fungicide rotation
- Only one triazole per season
- Only one strobilurin per season
- A good three spray program is triazole, tin, strobilurin
- Scout at end of the season to decide the necessity of a late application; CLS developed late in recent years
- NDAWN daily infection values, row closure, first appearance of disease and the calendar are all used to determine first fungicide application
- Use fungicide resistance maps for fungicide selection
- Use a variety with resistance to CLS; KWS rating of 5.0 or less
- Spray intervals of 14 days
- Use 15-20 gpa at 100-125 psi for ground application of fungicides and 5 gpa for air application

Fig 1. Sensitivity to TPTH of *C. beticola* isolates collected in ND and MN from 1998 to 2006 at 1.0 ppm as measured by bulk spore germination

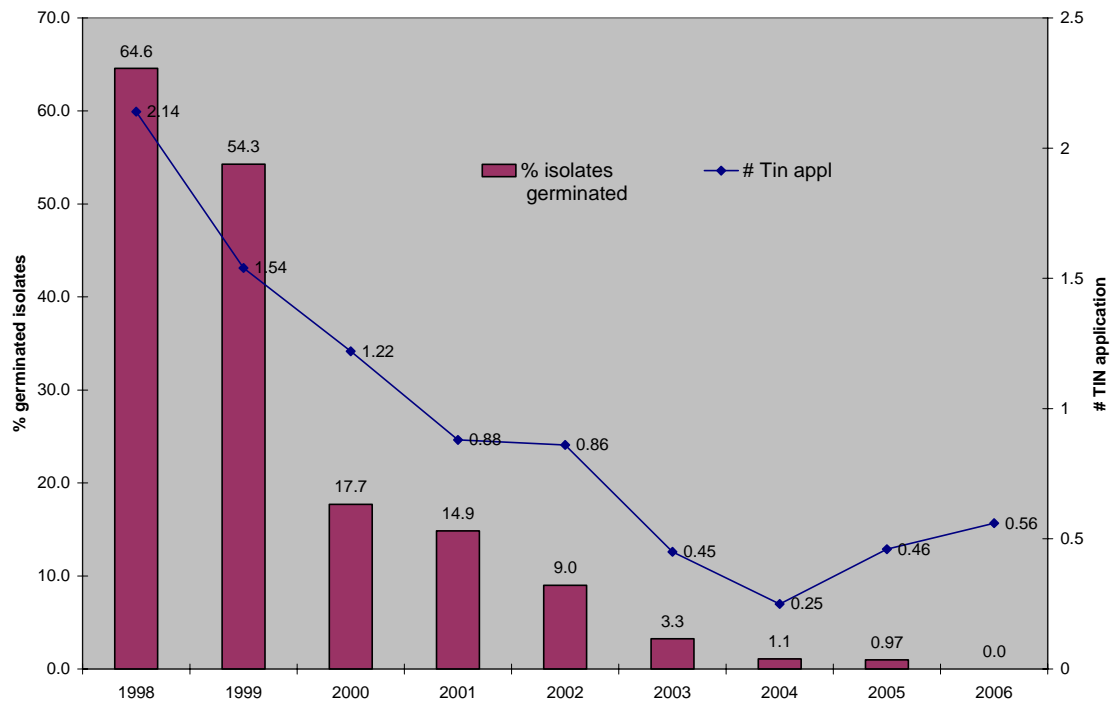


Fig 2. Average EC-50 value of *Cercospora beticola* isolates collected from 1997-2006 to tetraconazole.

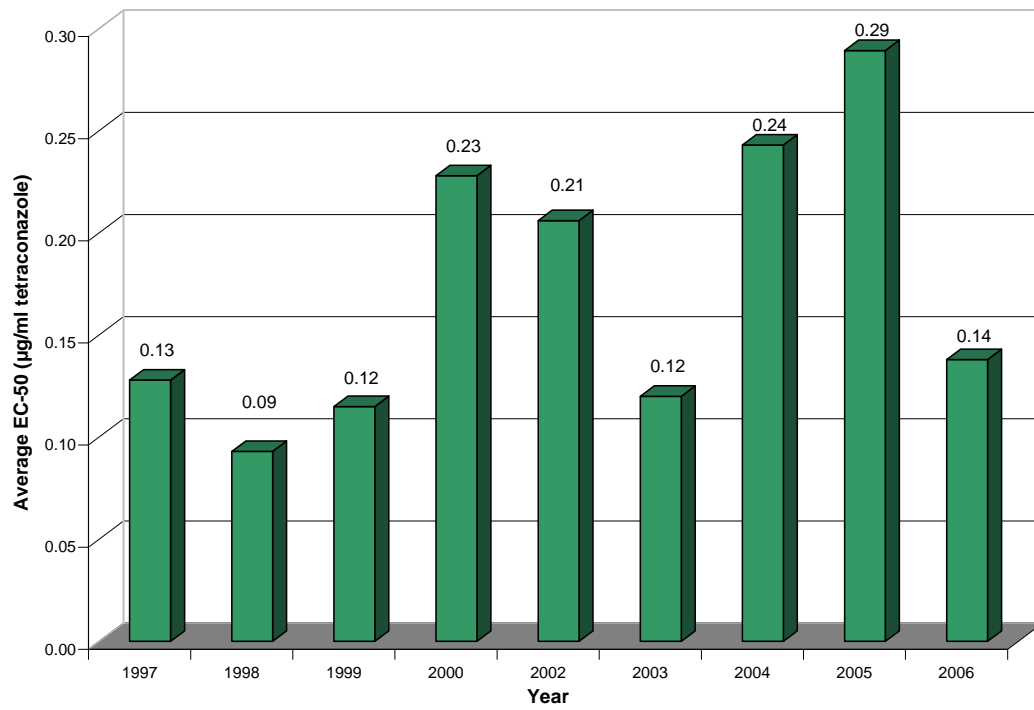


Fig. 3 Sensitivity of *C. beticola* isolates collected in ND and MN from 1997-2006 to tetraconazole

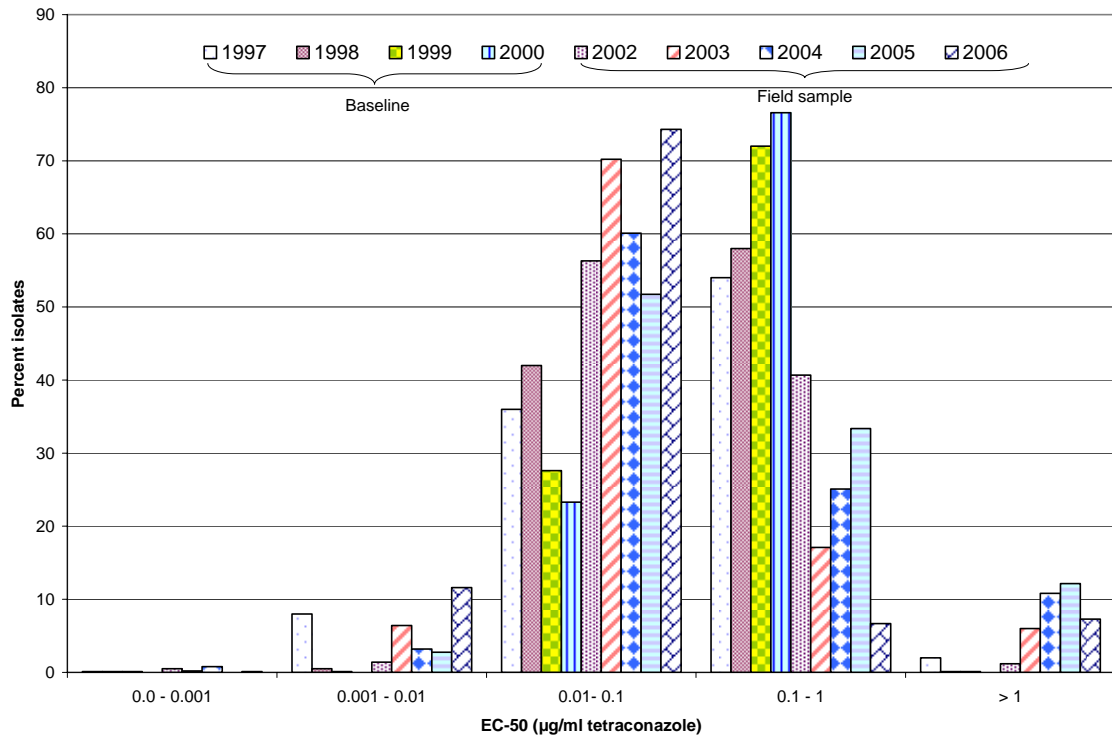


Fig 4. Sensitivity of *C. beticola* to tetraconazole by factory district 2005-2006

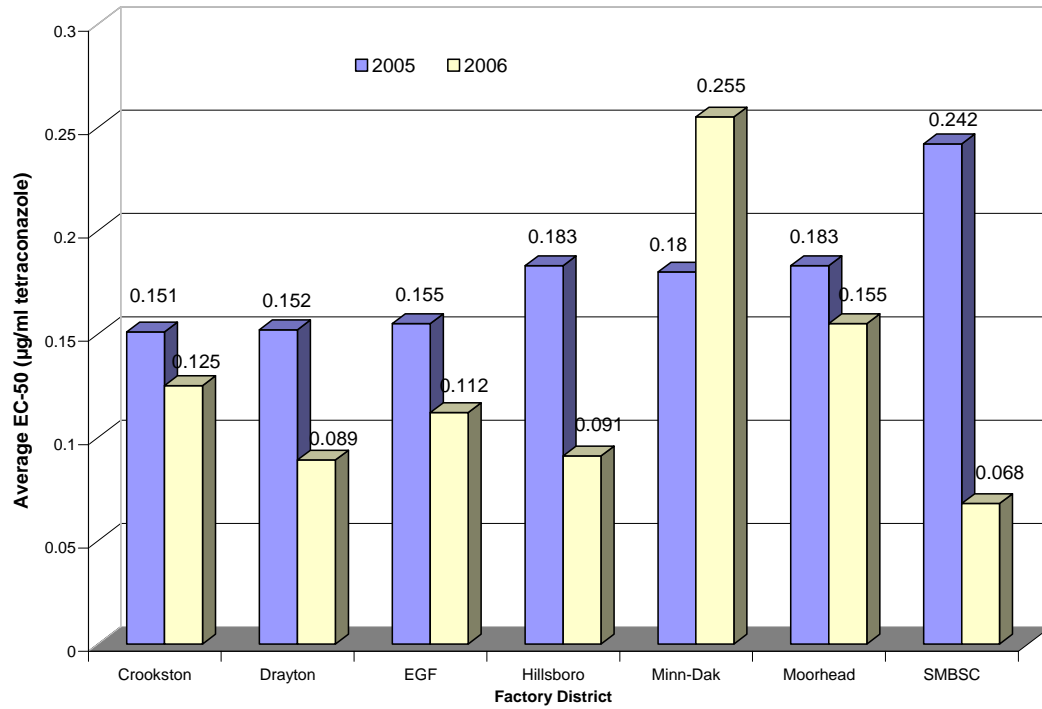


Fig 5. Percent of *C. beticola* isolates with EC-50 > 1 µg/ml of tetraconazole collected in 2006 by factory district

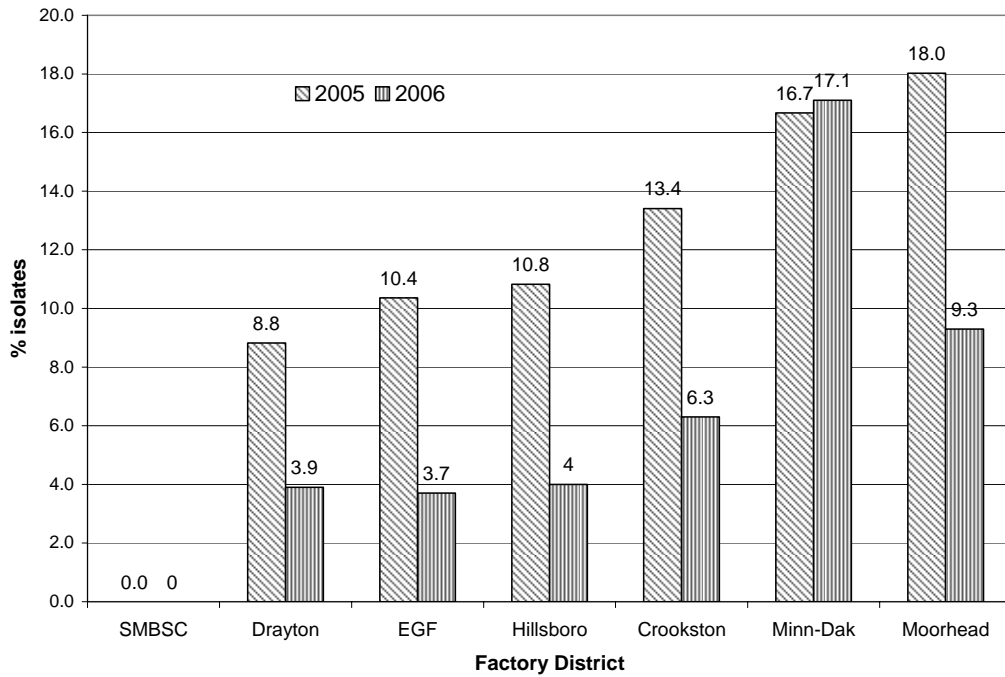


Fig 6. Sensitivity of *C. beticola* isolates to pyraclostrobin (Headline) collected from 2003-2006

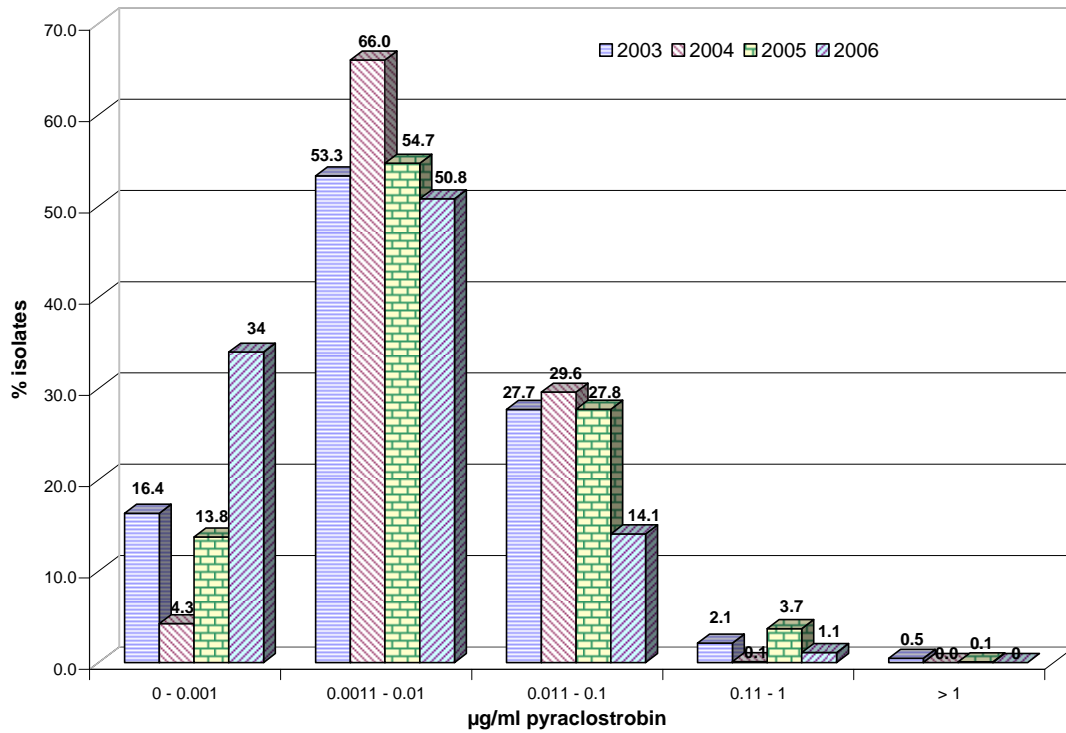


Fig 7. Sensitivity of *C. beticola* isolates collected in MN and ND to trifloxystrobin (Gem) from 2004-2006.

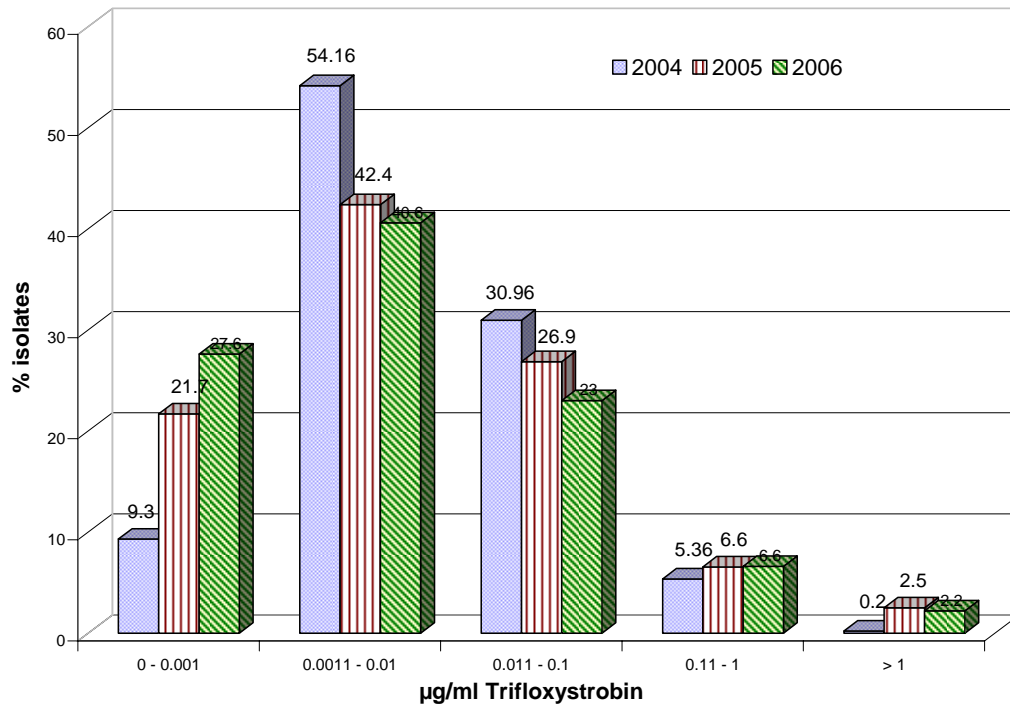


Fig. 8. Sensitivity of *C. beticola* isolates from ND and MN to Gem and Headline from 2003-2006 compared to the pre-registration baseline.

