SENSITIVITY OF *CERCOSPORA BETICOLA* TO FOLIAR FUNGICIDES IN 2008.

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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 Tin (triphenyl tin hydroxide), Topsin (thiophanate methyl), Eminent (tetraconazole), and Headline (pyraclostrobin). Tin is usually applied alone, but Topsin is usually applied as a tank mix with Tin. Recently Inspire and Proline were registered and are now being used.

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. It is important to monitor the *C. beticola* population for changes in sensitivity to these fungicides in order to achieve maximum disease control. We began testing *C. beticola* populations for sensitivity to tin in 1996, 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 these five commonly used fungicides in our area were conducted in the years 2003 - 2007. In 2008 sensitivity testing was conducted for tin, three triazole (DMI) fungicides, Eminent, Inspire, Proline, and one strobilurin (QoI) fungicide, Headline.

OBJECTIVES

The 2008 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 Tin (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) fungicide and compare sensitivity to previously established baseline.

3) Determine sensitivity of *Cercospora beticola* isolates from fields representing the sugarbeet production areas of ND and MN to two additional triazole (DMI) fungicides: difenaconazole (Inspire), and prothioiconazole (Proline).

4) Distribute results of sensitivity testing in a timely manner in order to make fungicide recommendations for disease management and fungicide resistance management based on test results.

METHODS AND MATERIALS

In 2008, with financial support of the Sugarbeet Research and Extension Board of ND and MN, Nufarm Amearicas, United Phosphorous, BASF Corporation, Syngenta Crop Protection 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, Inspire, Proline and Headline.

Sugar beet leaves with Cercospora leaf spot (CLS) were collected from commercial fields by agronomists from American Crystal Sugar Company, Minn-Dak Farmers Cooperative and Southern Minnesota Beet Sugar Cooperative representing all production areas in ND and MN. 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 per leaf from five leaves. The spores were mixed in water, and a composite of 200 µl of spores suspension was transferred to each of two Petri plates containing water agar amended with Tin at 1 ug/ml 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 triazole and Headline testing.

For triazole 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 triazole fungicide from 0.001 – 1.0 ppm. A separate test was conducted for each of the three triazole fungicides. 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 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 fungicide Headline, 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 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 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

Disease pressure was generally low and Cercospora disease again developed late in the 2008 season. The majority (87%) of the CLS samples were delivered to our lab in September. Due to the diligent collection efforts of the grower cooperative agronomists, 1141 field samples representing all production areas and factory districts were received. Of these A total of 899 *C. beticola* isolates were tested for sensitivity to five fungicides in 2008. An additional 113 samples from fungicide trial plots of Dr. Mohamed Khan (Foxhome), and 131 samples from the fungicide trial plots of Mark Bredehoft, SMBSC (Clara City) were also tested for sensitivity to these fungicides. For this report, only results from the field samples are included; the fungicide trial plot results are not included. A few samples that were submitted were not done, because the spores did not germinate. 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 Tin was first reported in 1994, with tolerance levels between 1-2 ppm. These levels reduced efficacy of control by tin. The incidence of Tin tolerance increased between 1997 and 1999, but incidence of isolates tolerant to tin at 1.0 ppm has been declining since the introduction of Eminent for resistance management in 1999, Gem in 2002 and Headline in 2003. In 1998, the percentage of isolates with tolerance to Tin 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%, in 2006 was 0.0%, in 2007 increased to 5.1%, and in 2008 was 0%. (Fig.1). Tin is once again an effective fungicide for managing CLS and an important partner for fungicide resistance management.

A baseline sensitivity curve was developed for Eminent using *C. beticola* isolates from 1997-1999 that had not been previously exposed to Eminent and the year 2000 from our culture collection. Compared to the baseline values there appears to be a slow increase in the average EC50 value of *C. beticola* isolates

from 1998 to 2005, but a decrease in EC50 values -n 2006-2008 (Fig 2). The average EC_{50} values of these *C. beticola* isolates from our culture collection are 0.13 (1997), 0.09 (1998), 0.12 (1999), and 0.23 (2000). The average EC_{50} value of field-collected isolates from 2002 was 0.21 ppm, from 2003 was 0.12 ppm, from 2004 was 0.24, and from 2005 was 0.29 (Fig. 2). There was a decline in the EC50 value in 2006 to 0.14, an increase in 2007 to 0.2, and in 2008 remains the same at 0.2 (Fig.2). These values include isolates with an EC_{50} value of >1.0 ug/ml.

In 2002, 1.2 % of the isolates tested had an EC_{50} 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 was for increased resistance to tetraconazole as indicated by an increase in both average EC_{50} values (Fig. 2) and the incidence of isolates with EC_{50} values >1 ppm (Fig. 3), but in 2006 there was a decrease in resistance to Eminent (Figs. 2 and 3).This reduction along with the reduction in Tin resistance, may indicate that our collective resistance management program and recommendations may be working. In 2008 a reduction in the average EC-50 value across all factory districts except for Minn-Dak which showed an increase in resistance. (Fig. 4). In 2007, the opposite was found; the lowest EC50 values were in the Minn-Dak area.

Sensitivity to two additional DMI (triazole) fungicides; difenaconazole (Inspire), and prothioiconazole (Proline) were tested. The average EC50 values of these two triazoles was Proline at 0.765 and Inspire 0.149 compared to Eminent at 0.21 µg/ml (Fig 5). The percent isolates highly resistant (>1.0 µg/ml) of the three triazoles was Proline 15.7%, Inspire 9.7% compared to Eminent at 12.4%. While the EC50 values of Proline are higher than either Eminent or Inspire, this is more of a reflection of intrinsic activity of the fungicide and does not indicate a higher level of resistance. The EC50 values of Proline decreased in 2008, while the EC50 values for Eminent and Inspire remained basically unchanged (Fig. 5).

Baseline sensitivity to the QoI (strobulurin) fungicide Headline was calculated using *C. beticola* isolates from our culture collection that were not previously exposed to Headline. This baseline is used to monitor shifts in sensitivity to this fungicide. Sensitivity of C. *beticola* to Headline has remained relatively stable from 2003-2008 with only an 8-10 fold decrease in sensitivity compared to the baseline (Fig. 6). It should be emphasized that we have found isolates in the population that have an EC_{50} value >1.0 ppm (a 400 fold decrease in sensitivity) for both Headline. In 2008, an increase in the number of isolates with an EC-50 > than 0.001 µg/ml was observed, from 48.8% in 2007 to 53% in 2008, and a decrease in the percent of isolates with an EC-50 < than 0.001, from 26.8% in 2007 to a 21.8% in 2008 (Fig. 7). It is important to know that there are numerous examples in many crops where resistance has developed to strobilurin (QOI) fungicides due to over application and misapplication of these fungicides. Because of the widespread application of Headline to sugar beets at the end of the season, the application to most other crops in the sugar beet production area, and the potential for resistance development, it remains critical to monitor sensitivity of *C. beticola* to Headline.

Because *C. beticola* has a history of developing resistance to fungicides, and has a high degree of variablility in culture, the potential for resistance development to fungicides is always there. This is especially true since we found both mating types of *C. beticola* naturally occurring in the population in ND and MN. 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 high priority.

SUMMARY

1. Tin tolerance at 1.0 ppm has almost disappeared in our region, because of 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. In 2008 no resistant isolates were found.

2. Sensitivity to Eminent is relatively stable, but there has been 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. In 2006 for the first time since testing began, there was a decrease in both the number of isolates with an EC_{50} value >1.0 ppm and the overall EC_{50} value across all isolates tested. In 2008, a decrease in resistance to Eminent was observed in all factory districts except Minn-Dak.

3. Sensitivity to Headline remains relatively stable, but there are rare isolates identified with a 400fold decrease in sensitivity. There has been a slight change in sensitivity (approximately 10X) to Headline compared to the baseline since use and testing began five years ago. This change is not a cause for concern, but a few resistant isolates > 1 ppm were found in the survey which has the potential for concern.

5. It appears that the fungicide resistance management plan that we are following is working since resistance is stable since there have been no fungicide failures in our area due to fungicide resistance.

6. Disease pressure has been low, and higher disease pressure may change fungicide sensitivity patterns.

7. 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.

8. 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 2008 at 1.0 ppm as measured by bulk spore germination

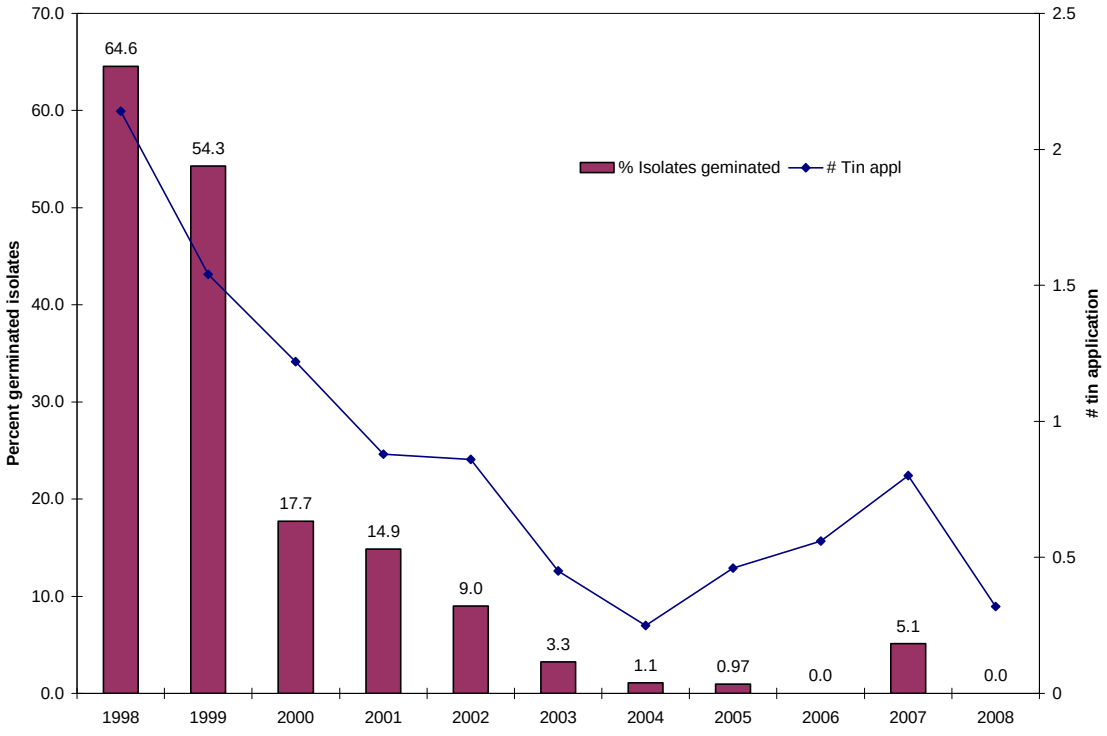

Fig 2. Average EC-50 value of *Cercospora beticola* isolates collected from 1997-2008 to tetraconazole.

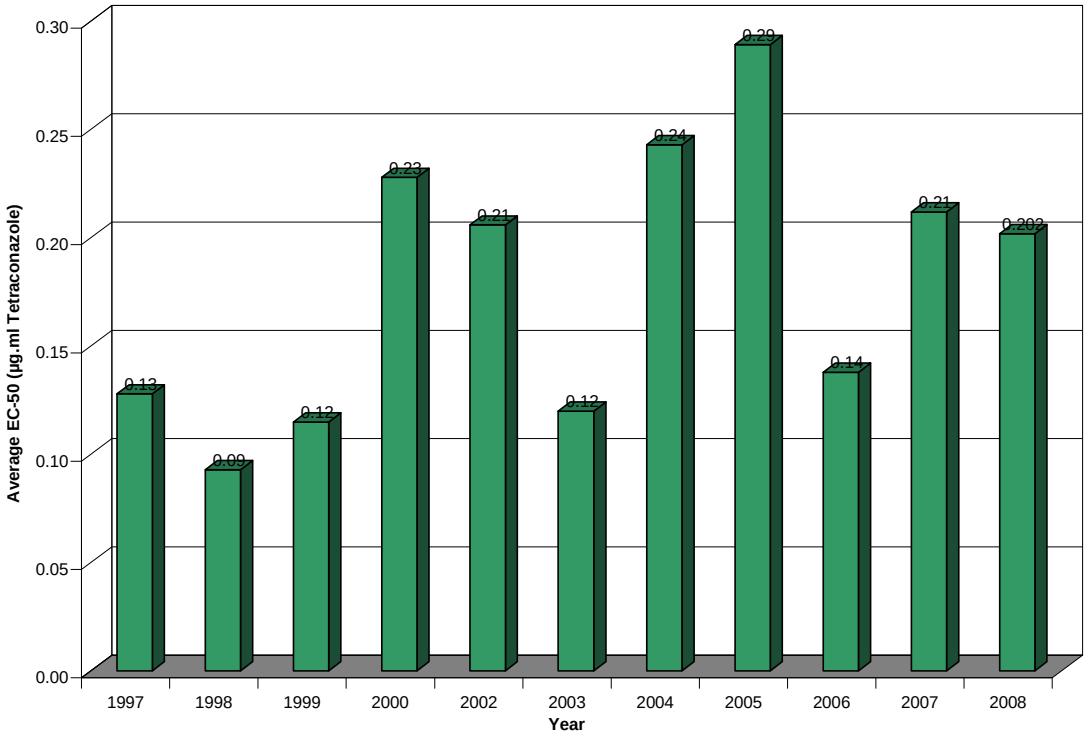

Fig.3 Sensitivity of *C. beticola* isolates collected in ND and MN from 1997-2008 to tetraconazole

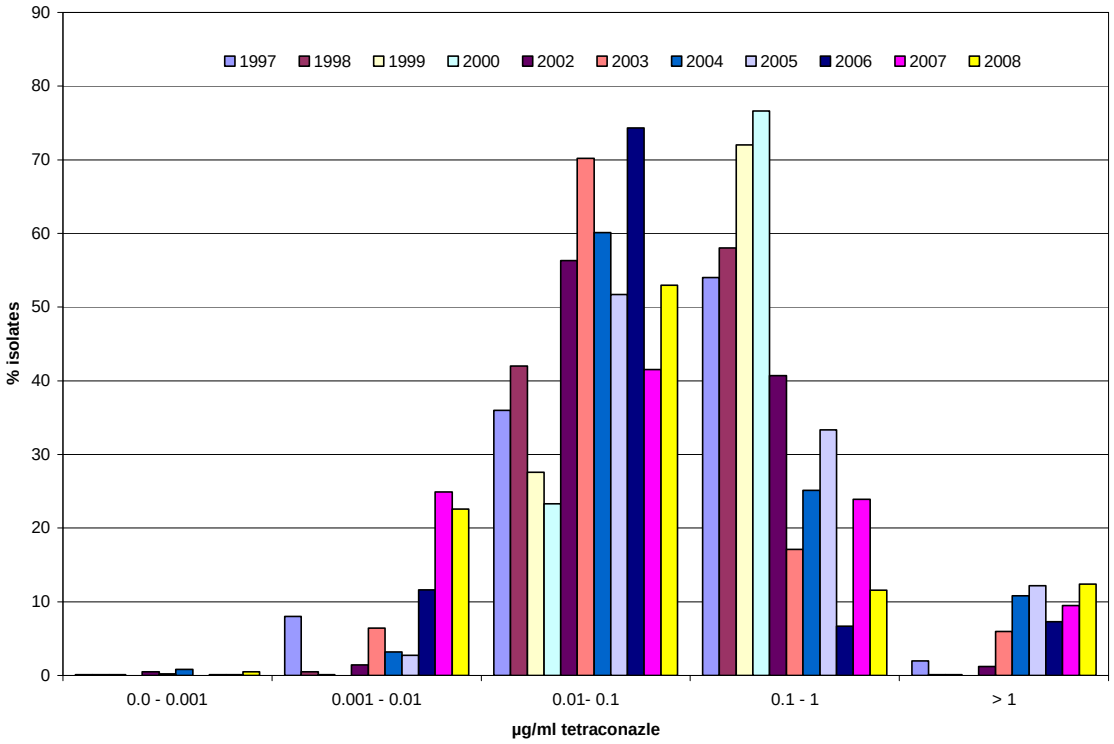

Fig 4. Sensitivity of *C. beticola* to tetraconazole by factory district 2005-2008

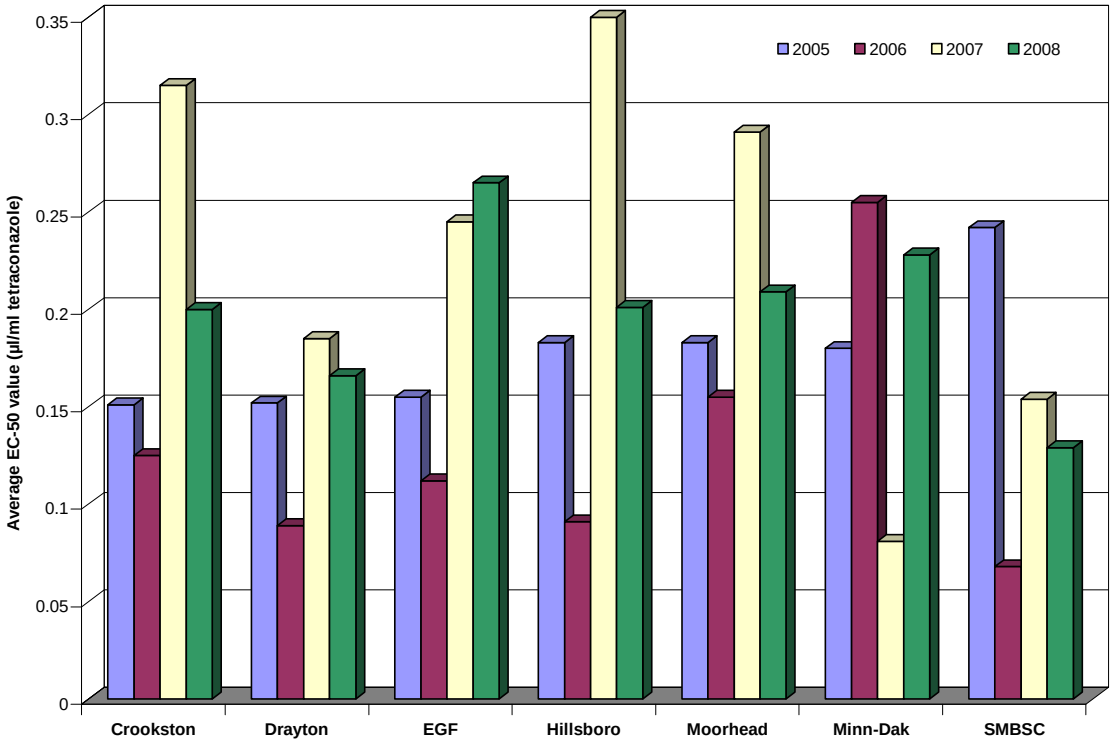

Fig 5. EC-50 values of C. beticola isolates collected in 2007-2008 to three triazole fungicides

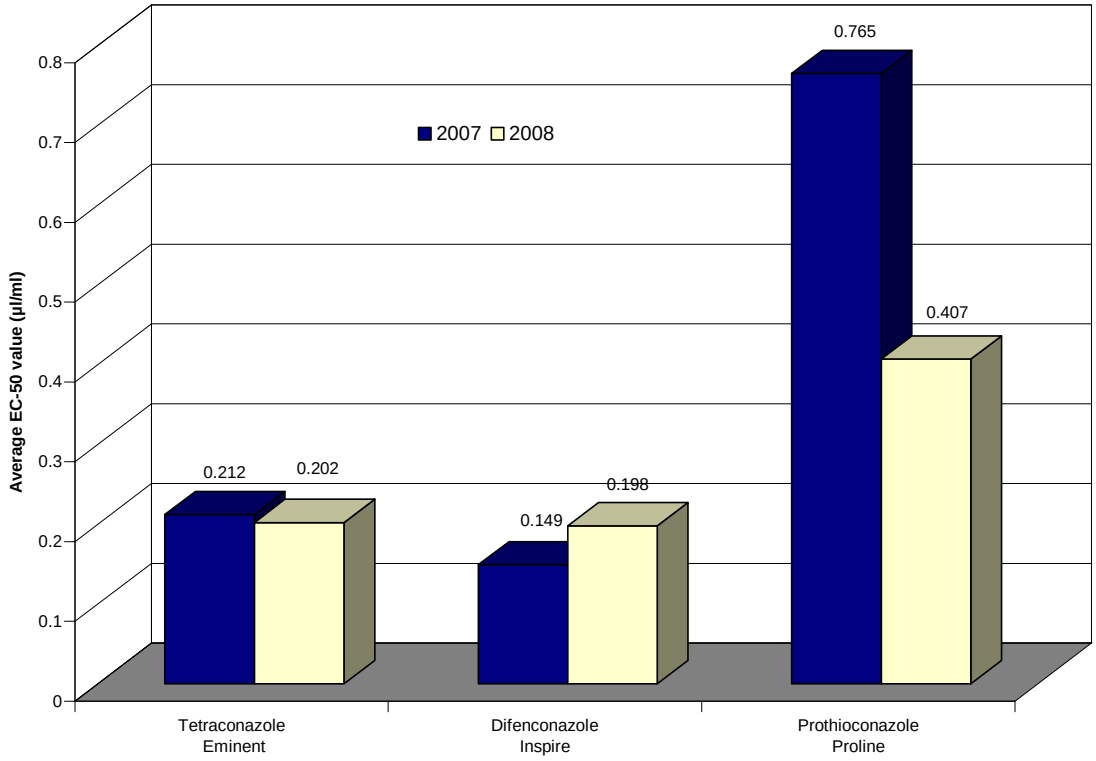

Fig. 6. Average EC-50 (µg/ml) values of *C. beticola* isolates collected in ND and NM to pyraclostrobin (Headline) from 2003 to 2008

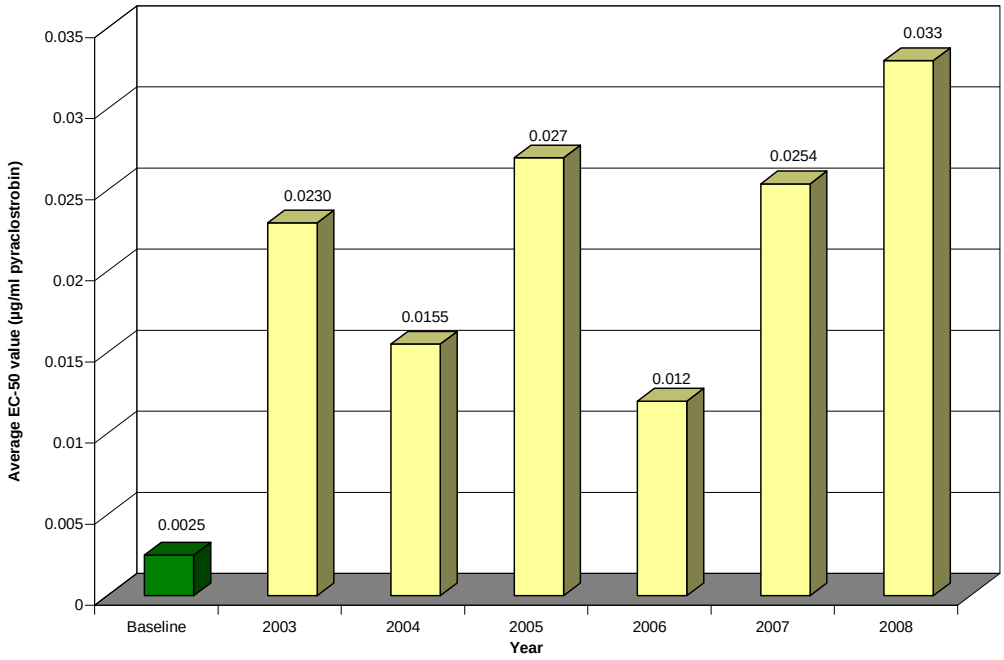

Fig. 7 Sensitivity of *C. beticola* isolates collected in ND and MN from 2003 to 2008 to pyraclostrobin (Headline)