SENSITIVITY OF *CERCOSPORA BETICOLA* TO FOLIAR FUNGICIDES IN 2007.

Gary Secor, Viviana Rivera and Mohamed Khan
Department of Plant Pathology, North Dakota State University, Fargo, ND 58105 USA

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), Gem (trifloxystrobin) and, Headline (pyraclostrobin). Tin is usually applied alone, but Topsin is usually applied as a tank mix with Tin.

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 has been conducted in the years 2003 - 2006. In 2007 sensitivity testing was done for tin, Topsin, four triazole (DMI) and two strobilurin (QoI) fungicides.

OBJECTIVES

The 2007 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), Topsin (thiophanate methyl) 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) Determine sensitivity of *Cercospora beticola* isolates from fields representing the sugarbeet production areas of ND and MN to three additional triazole (DMI) fungicides: fenbuconazole (Enable), difenaconazole (Inspire), and prothioiconazole (Proline).

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

METHODS AND MATERIALS

In 2007, with financial support of the Sugarbeet Research and Extension Board of ND and MN, Sipcam Agro, BASF Corporation, Dow AgroSciences, 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, Topsin, Eminent, Enable, Inspire, Proline, Headline and Gem.

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 µl of spores transferred to each of two Petri plates containing water agar amended with Tin at 1 ug/ml or non-amended (water agar

alone). For every third sample received, a composite of spores was also transferred to a Petri dish containing water agar amended with 5 ug/ml of Topsin.

For Tin and Topsin 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 and other triazoles, Headline and Gem sensitivity testing.

For Eminent and other 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 ug/ml. 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 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 ug/ml. 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). 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 again developed late in the 2007 season and the majority (86%) of the CLS samples were delivered to our lab in September. A total of 1438 *C. beticola* isolates were tested for sensitivity to eight fungicides in 2007. Due to the diligent collection efforts of the grower cooperative agronomists, 1026 field samples representing all production areas and factory districts were received and tested. An additional 412 samples from fungicide trial plots of Dr. Mohamed Khan (Foxhome) and Mark Bredehoeft (Renville) were also tested for sensitivity to these fungicides. For this report, only results from the field samples are included; the fungicide trial results are not included. A few samples that were submitted 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 Tin was first reported in 1994, with tolerance levels between 1-2 ug/ml. The incidence of Tin tolerance increased between 1997 and 1999, but incidence of isolates tolerant to Tin at 1.0 ug/ml 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 ug/ml 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%, and in 2007 increased to 5.1%. (Fig.1). Percent tin sensitivity by factory district was Crookston 0, Drayton 5.1, EGF 3.6, Hillsboro 3.1, Moorhead 6.7, MinDak 13.4 and SMBSC (Fig. 2).

Resistance to the benzimidazole fungicide Topsin 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 Topsin at >5.0 ug/ml 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, 69.3% of

the samples were resistant; and in 2004, 78.3% of the isolates were resistant. Due to the widespread resistance to Topsin sensitivity to Topsin was not tested in 2005 or 2006, but was tested in 2007. Overall, 42.0 percent of the samples were resistant to Topsin at 5 ug/ml. Sensitivity to Topsin declined in most factory districts; the percent isolates resistant to Topsin by factory district was: Crookston 0, Drayton 35.4, EGF 39.3, Hillsboro 67.6, Moorhead 77.8, MinDak 48.1, SMBSC 25.0 (Fig 3). It appears that resistance to Topsin continues to be present in most of the sugarbeet production area of North Dakota and Minnesota and but is declining in most factory districts. Topsin is only recommended as a tank mix partner with Tin.

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. 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 ug/ml, from 2003 was 0.12 ug/ml, from 2004 was 0.24, and from 2005 was 0.29 (Fig. 4). There was a decline in the EC50 value in 2006 to 0.14, and an increase in 2007 to 0.21 (Fig.4). 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 5). The trend from 2003 - 2005 was for increased resistance to tetraconazole as indicated by an increase in both average EC_{50} values (Fig. 6) and the incidence of isolates with EC_{50} values >1 ug/ml (Fig. 5), but in 2006 there was a decrease in resistance to Eminent (Figs. 5 and 6).This reduction along with the reduction in Tin resistance, may indicate that our collective resistance management program and recommendations may be working. In 2007 there was an increase in resistance to Eminent across all factory districts except for MinDak which showed a three-fold reduction in resistance (Fig. 6).

Sensitivity to three additional DMI (triazole) fungicides; fenbuconazole (Enable), difenaconazole (Inspire),and prothioiconazole (Proline) was also conducted in 2007. The average EC50 values of these three triazoles was Proline (0.77), Enable (0.37), Inspire (0.15) compared to Eminent at 0.21 (Fig 7). The percent isolates highly resistant (>1.0 ug/ml) of the three triazoles was Proline (37.5), Enable (9.7), Inspire (5.4) compared to Eminent at 9.5 (Fig. 8).

Baseline sensitivity to the QoI (strobilurin) fungicides Headline and Gem was calculated using *C. beticola* isolates from our culture collection that were not previously exposed to Headline and Gem. This baseline will be used to monitor shifts in sensitivity to these fungicides. Sensitivity of C. *beticola* to both of these fungicides has remained relatively stable from 2003-2007 with only an 8-10 fold decrease in sensitivity compared to the baseline (Figs. 9) since these fungicides have been used commercially (Headline since 2003, Gem since 2004). However, substantial variability exists among the isolates tested, with a thousand-fold difference in EC_{50} values among the isolates to pyraclostrobin and trifloxystrobin, indicating the potential for reduced sensitivity is present in the population (Figs. 10 and 11). It should be emphasized that we have found isolates in the population that have an EC_{50} value >1.0 ug/ml 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 over application 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 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 priority.

SUMMARY

1. Tin tolerance at 1.0 ug/ml has almost 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. However in 2007 a slight increase was noted in most factory districts.

2. Resistance to Topsin at 5.0 ug/ml is present in most production areas in 2007, but appears to be declining in some areas.

3. Sensitivity to Eminent is relatively stable, but there has been a slow increase in the number of isolates with an EC_{50} > 1.0 ug/ml 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 ug/ml and the overall EC_{50} value across all isolates tested. However in 2007, there was an increase in resistance to Eminent in all factory districts except MinDak.

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. There have been not fungicide failures in our area due to resistance to fungicides.

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

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 2007 at 1.0 ug/ml as measured by bulk spore germination

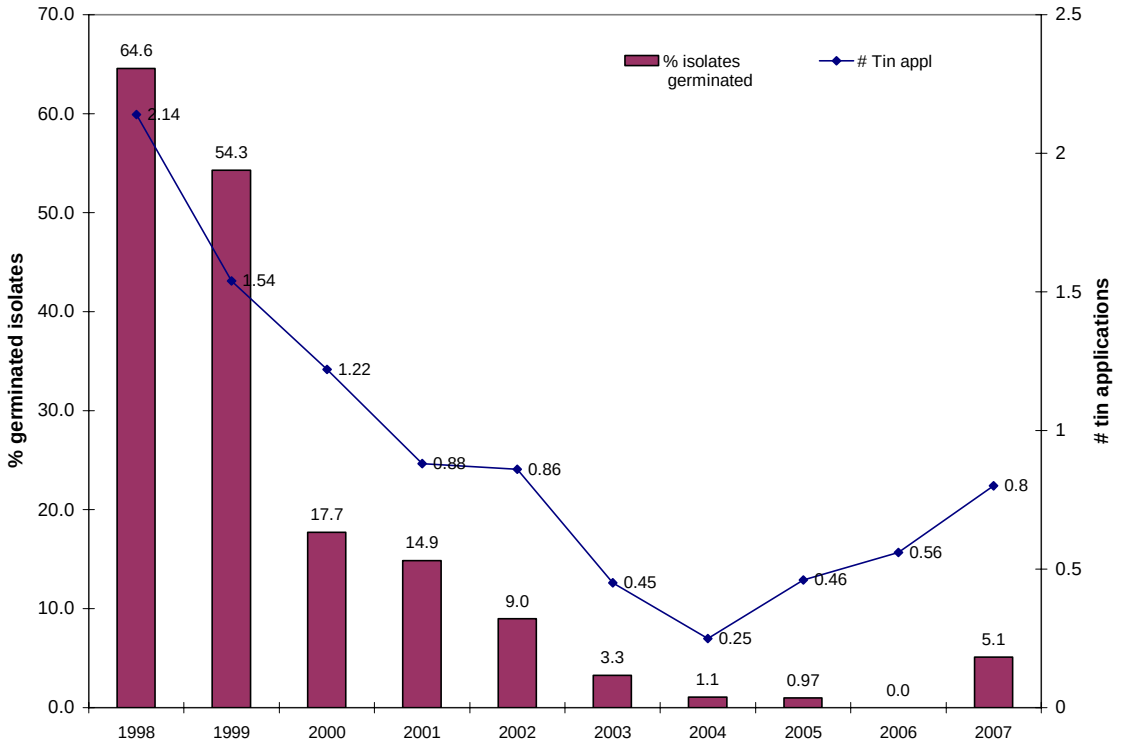

Fig. 2 Percent of *C. beticola* isolates collected in 2007 resistant to Tin by factory district

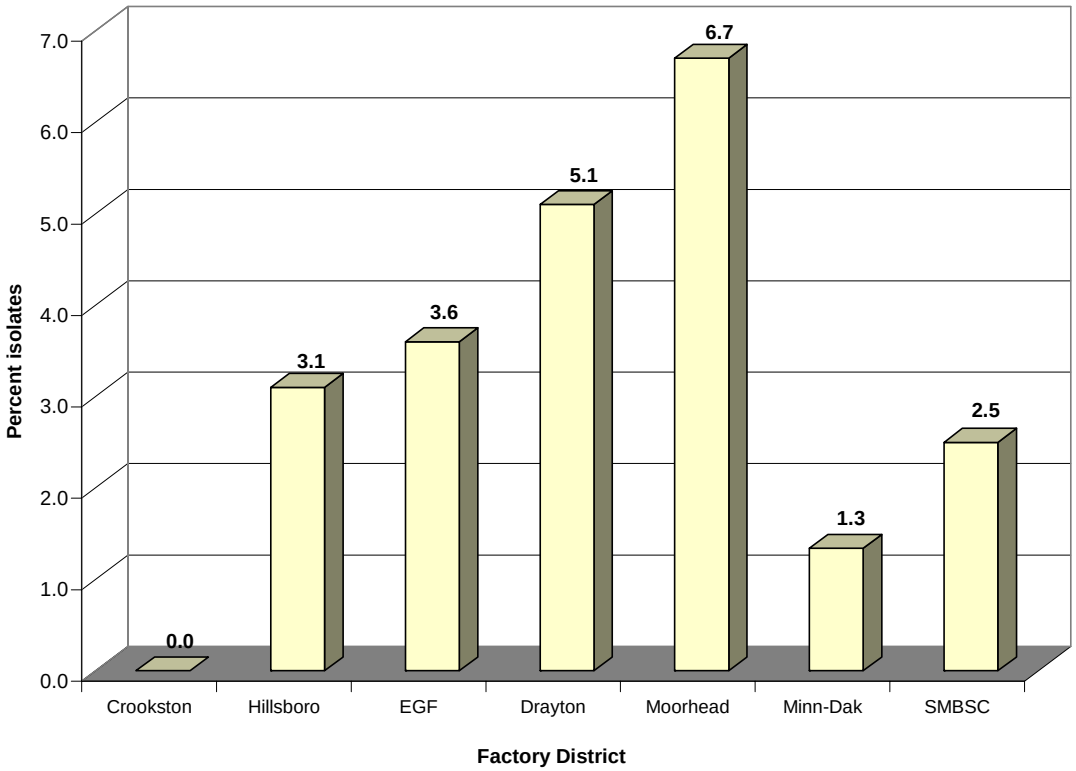

Fig. 3. Percent of *C. beticola* isolates resistant to Topsin (5 µg/ml) by factory district in 2007

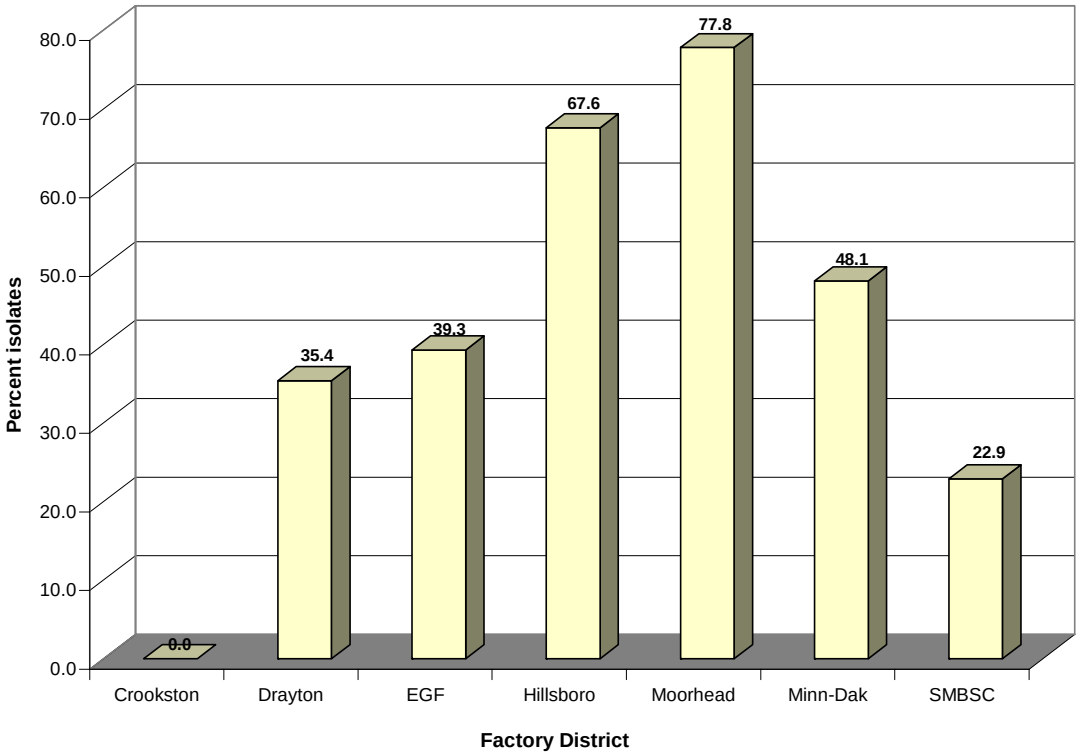

Fig 4. Average EC-50 value of *Cercospora beticola* isolates collected from 1997-2007 to tetraconazole.

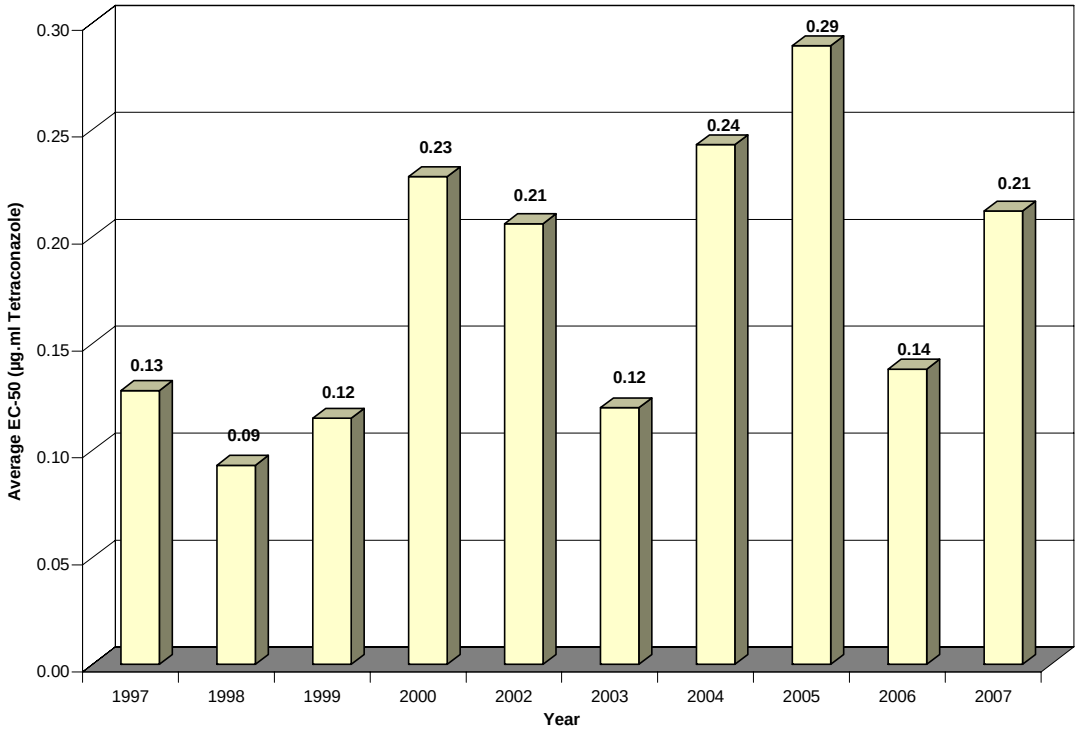

Fig. 5 Sensitivity of *C. beticola* isolates collected in ND and MN from 1997-2008 to tetraconazole

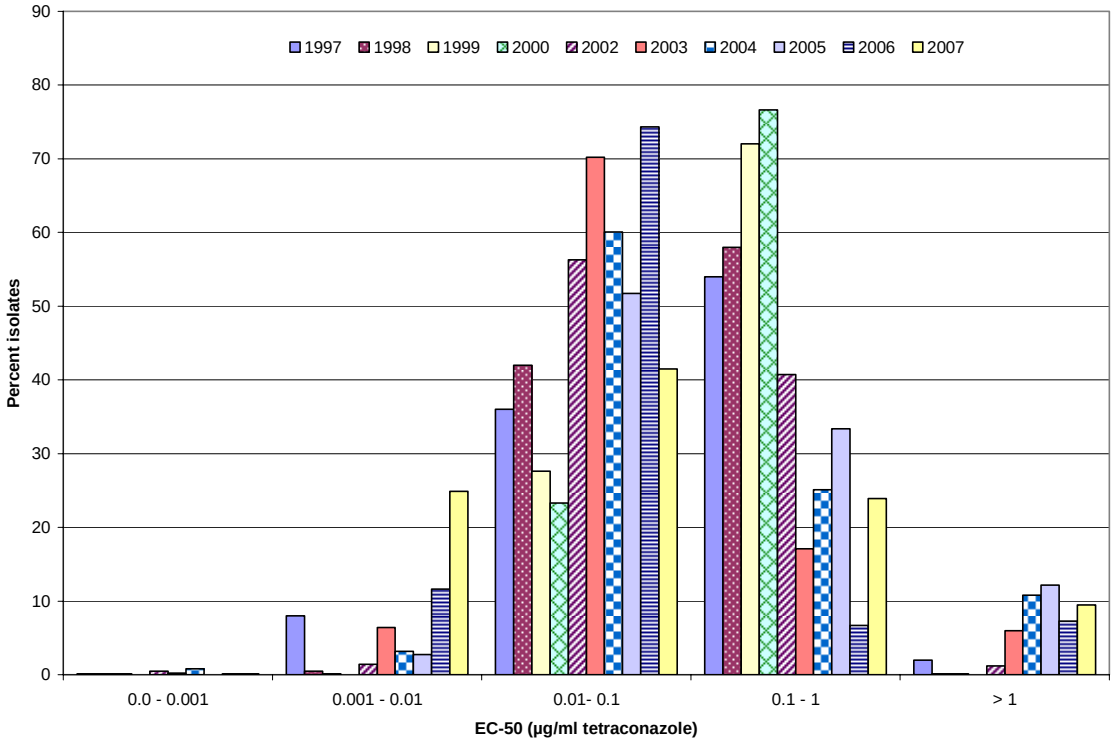

Fig 6. Sensitivity of *C. beticola* to tetraconazole by factory district 2005-2007

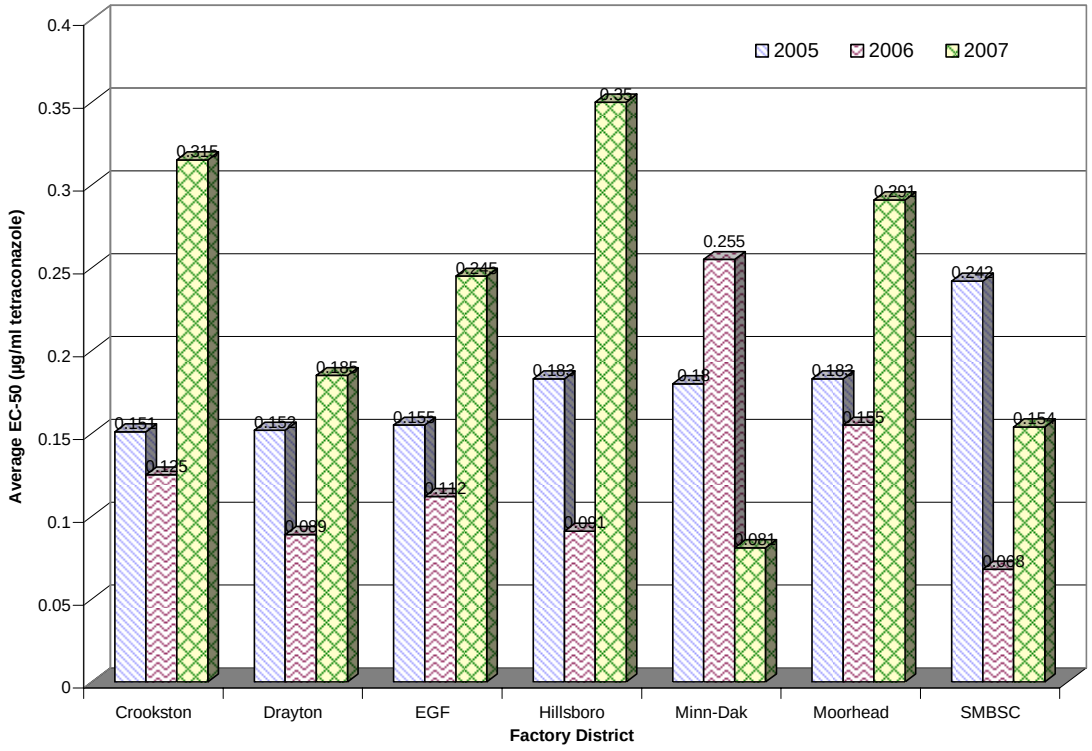

Fig 7. EC-50 values of C. beticola isolates collected in 2007 to four triazole fungicides

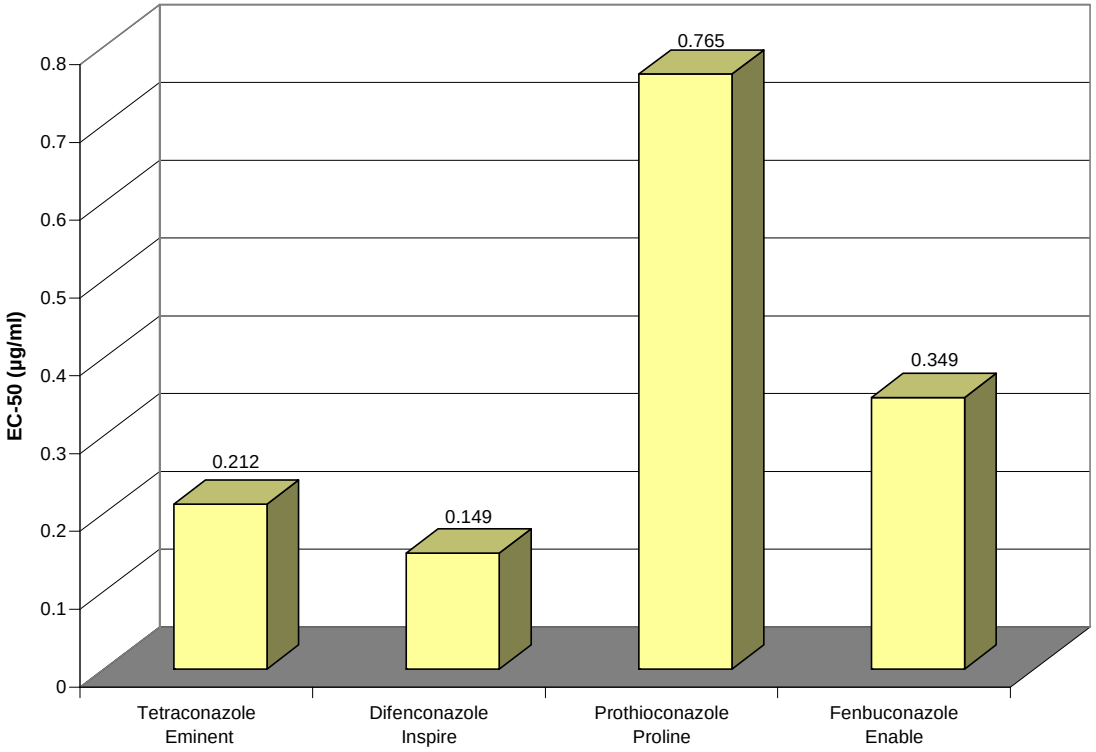

Fig 8. Percent *C. beticola* isolates with a EC-50 > 1 µg/ml for four triazole collected in 2007

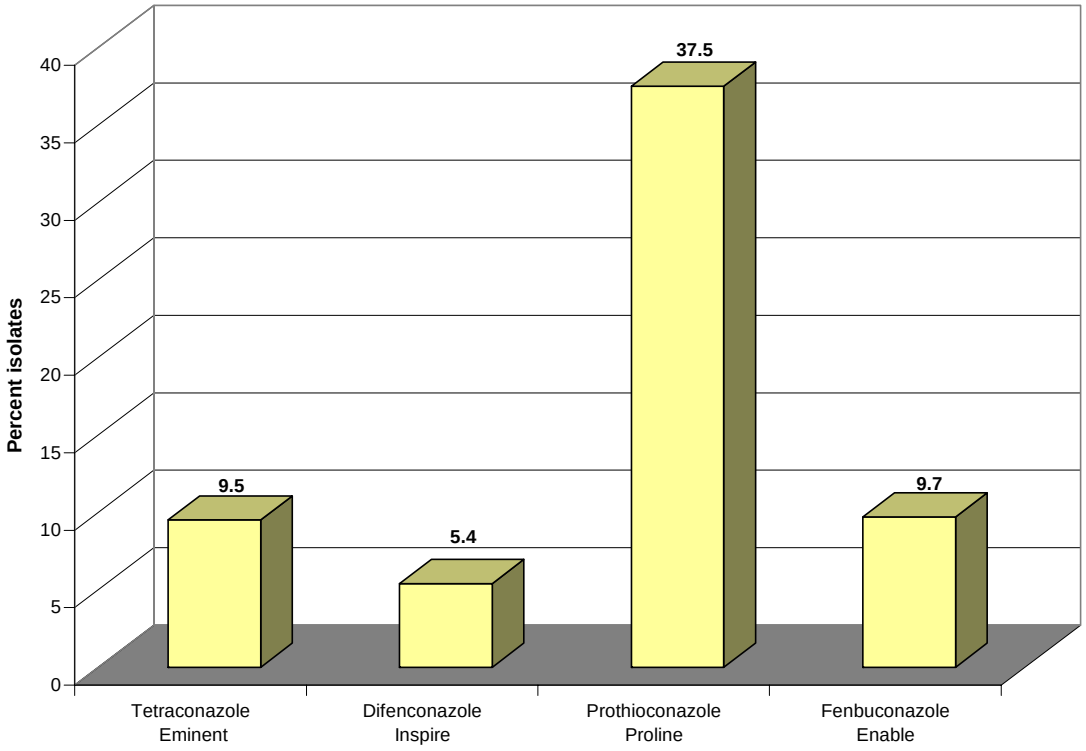

Fig. 9. Average EC-50 (µg/ml) values of *C. beticola* isolates collected in ND and NM to pyraclostrobin (Headline) and trifloxystrobin (Gem) from 2003 to 2007

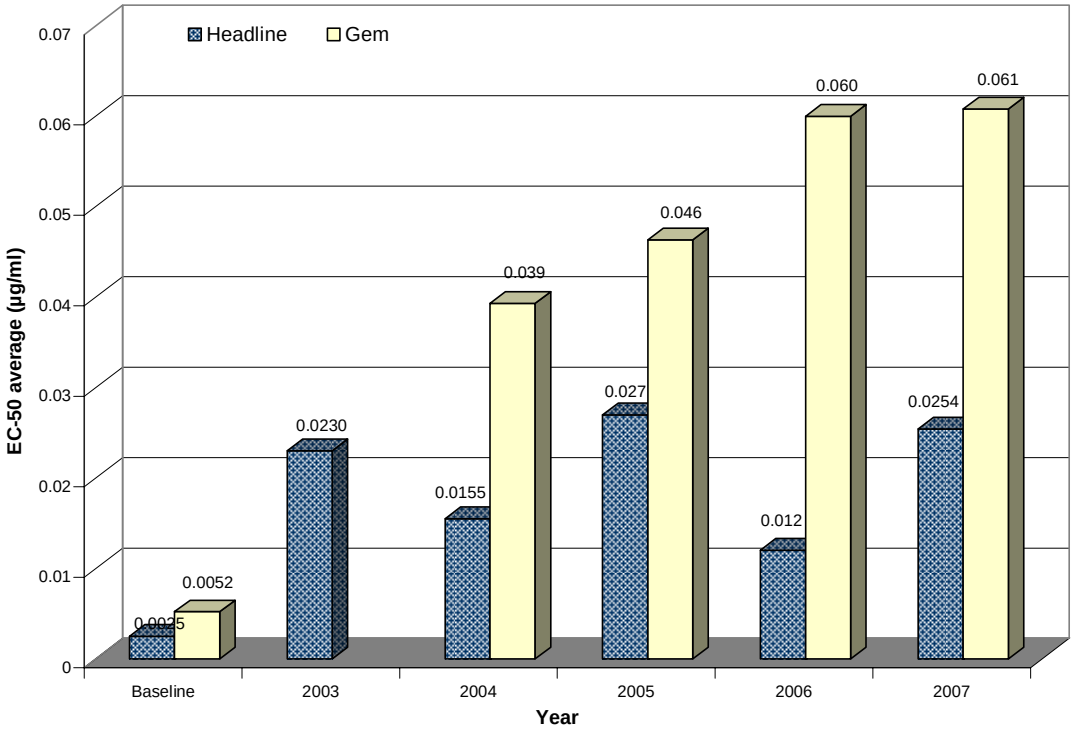

Fig. 10 Sensitivity of *C. beticola* isolates collected in ND and MN from 2003 to 2007 to pyraclostrobin (Headline)

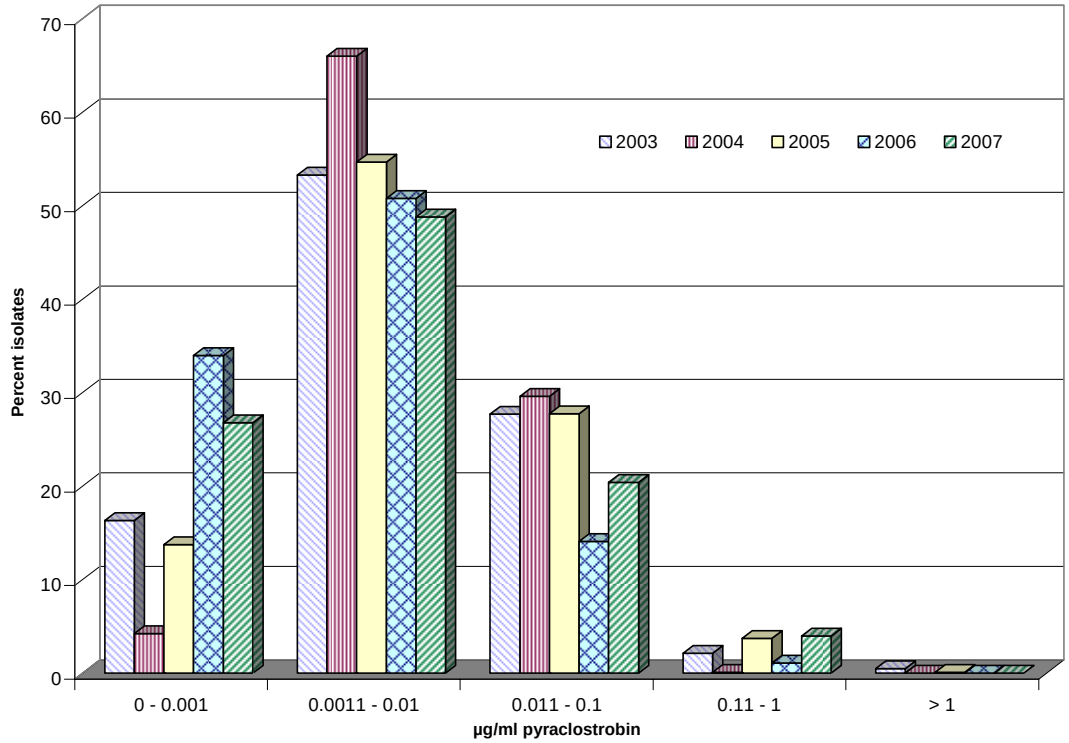

Fig 11. Sensitivity of Cercospora beticola isolates collected in 2004 to 2007 to trifloxystrobin (Gem)