SENSITIVITY OF *CERCOSPORA BETICOLA* TO FOLIAR FUNGICIDES IN 2009

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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 used at high label rates and are alternated. The most frequently used fungicides are Tin (triphenyl tin hydroxide), Topsin (thiophanate methyl), Eminent (tetraconazole), Proline (prothioconazole), Inspire (difenoconazole) and Headline (pyraclostrobin). All fungicides are applied alone, except Topsin, which is usually applied as a tank mix with Tin.

Like many other fungi, *C. beticola* has the ability to adapt to repeated fungicide exposure and become less sensitive to the fungicides used to control them, especially if they are applied frequently over a period of time. Loss of disease control can result when fungicides become less sensitive. It is important to monitor the *C. beticola* population for changes in sensitivity to the fungicides used for Cercospora leaf spot management in order to achieve maximum disease control. We began testing *C. beticola* populations for changes in sensitivity to tin in 1996, and expanded sensitivity testing to additional fungicides in subsequent years. From 1997-2000 we evaluated sensitivity of *C. beticola* 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 commonly used fungicides in our area was conducted in the years 2003 - 2008. In 2009 sensitivity testing was conducted for tin, Topsin, three triazole (DMI) fungicides, Eminent, Inspire, Proline, and one strobilurin (QoI) fungicide, Headline.

OBJECTIVES

The 2009 objectives were:

1) 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 Topsin (thiophanate methyl).

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

3) Determine sensitivity of *Cercospora beticola* isolates from fields representing the sugarbeet production areas of ND and MN to three triazole (DMI) fungicides: Eminent (tetraconazole), Inspire (difenoconazole), and Proline (prothioconazole)).

4) Distribute results of sensitivity testing in a timely manner to the sugar beet industry in order to make fungicide recommendations for disease management and fungicide resistance management for Cercospora leaf spot disease in our region.

METHODS AND MATERIALS

In 2009, with financial support of the Sugarbeet Research and Extension Board of MN and ND, Nufarm Americas, 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, Topsin, Eminent, Inspire, Proline and Headline. For this report we use the commercial name of the fungicides, but all testing was conducted using the technical

grade active ingredient of each fungicide, not the formulated commercial fungicide. The term µg/ml is equivalent to ppm.

Sugar beet leaves with Cercospora leaf spot (CLS) were collected from commercial sugar beet 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 the spore suspension was transferred to each of three Petri plates containing water agar amended with Tin at 1 ug/ml, amended with Topsin at 5 µg/ml or non-amended (water agar alone).

For Tin and Topsin sensitivity testing, a bulk spore germination procedure was used. Germination of 100 random spores on the Tin and Topsin 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, Proline, Inspire and Headline testing.

For triazole fungicide sensitivity testing, a standard radial growth procedure 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 each 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 of *C. beticola* on non-amended water agar medium. This data was used to calculate an EC_{50} value for each isolate; EC_{50} is a standardized method of measuring fungicide resistance and is calculated by comparing the concentration of fungicide that reduces radial growth of *C. beticola* by 50% compared to the growth on non-amended media. Higher EC_{50} values mean reduced sensitivity to the fungicide.

For Headline sensitivity testing we 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. The spores are collected and transferred to water agar amended with serial ten fold dilutions of technical grade pyraclostrobin from 0.001 – 1.0 ppm plus SHAM. 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 a standardized method of measuring fungicide resistance and is calculated by comparing the concentration of fungicide that inhibits the germination of *C. beticola* by 50% compared to germination on non-amended media. Higher EC_{50} values mean reduced sensitivity to the fungicide.

RESULTS AND DISCUSSION

In 2009, disease pressure was generally low and Cercospora disease again developed late in the season. The majority of the CLS samples were delivered to our lab in late August and September. Approximately 480 field samples representing all production areas and factory districts were received. Of these, 456 *C. beticola* isolates were tested for sensitivity to six fungicides in 2009. Additional samples from fungicide trial plots of Dr. Mohamed Khan, NDSU (Foxhome), and 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 leaves collected had bacterial leaf spot and not Cercospora leaf spot.

Tolerance (resistance) to Tin was first reported in 1994 at concentrations of 1-2 µg/ml. At these levels, disease control in the field was reduced. The incidence of isolates with resistance to Tin at1.0 µg/ml increased between 1997 and 1999, but the incidence of resistant isolates has been declining since the introduction of additional fungicides for resistance management, including Eminent in 1999, Gem in 2002

and Headline in 2003. In 1998, the percentage of isolates resistant to Tin at 1.0 µg/ml was 64.6%, in 1999 was 54.3%, in 2000 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 was 5.1%, in 2008 was 0% and in 2009 was 2.0% (**Figure 1**).The incidence of isolates with resistance to Tin continues to be low, and Tin is an important component of fungicide resistance management program.

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 µg/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, 71.3% of the samples were resistant; in 2004, 78.3% of the isolates were resistant, and in 2009, 14% of the samples were resistant (**Figure 2**). It appears that incidence of isolates resistant to Topsin has decreased dramatically since last tested in 2004. This reduction is somewhat surprising since resistance to benzimidazole fungicides does not revert to sensitive quickly. We do not know the reason for the reduction in resistance, but we do know that with other benzimidazole fungicides, resistance may quickly return in the population when application of fungicides in this class resume.

Based on average EC_{50} values, overall resistance of *C. beticola* isolates to Eminent has doubled from 1998 to 2009 (**Figure 3**). The average EC_{50} value of field-collected isolates collected in 2002 was 0.21µg/ml, in 2003 was 0.12, in 2004 was 0.24, in 2005 was 0.29, in 2006 was 0.14, in both 2007 and 2008 was 0.20, and in 2009 was 0.25. In 2002, 1.2 % of the isolates tested had an EC_{50} value of >1 compared to 6.0% of the isolates in 2003, 10.8% of the isolates in 2004, 12.4% of the isolates in 2005, 7.3% of the isolates in 2006, 9.5% of the isolates in 2007, 12.4% of the isolates in 2008, and 6.6% of the isolates in 2009 .

Sensitivity to two additional triazole (DMI) fungicides Inspire), and Proline was also tested, but only from 2007-2009. The average EC_{50} values of prothioconazole were 0.77 in 2007, 0.41 in 2008, and 0.35 in 2009 (**Figure 4**). The average EC_{50} values for Inspire were 0.15 in 2007, 0.20 in 2008 and 0.10 in 2009 (**Figure 4**). In 2009, the percent isolates in 2009 highly resistant (>1.0 µg/ml) to Proline was 8.2% and to Inspire was 0.5%, compared to Eminent at 6.6%. 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 necessarily indicate a higher level of resistance. The EC_{50} values of Proline decreased in 2008, while the EC_{50} values for Eminent and Inspire remained basically unchanged.

Figure 5 shows the sensitivity of C. beticola to all these three triazole fungicides by factory district in 2009. The trend from 2003 - 2009 was for stable resistance to tetraconazole as indicated by both average EC_{50} values and the incidence of isolates with EC_{50} values >1 µg/ml. With the exception of Proline in 2007, resistance values to Eminent, Proline and Inspire are stable from 2007-2009 (**Figure 4**). This stability, along with the reduction in Tin resistance, may indicate that our collective resistance management program and recommendations are working. However, we do not know at what EC_{50} value disease control is reduced in the field. Additional work is necessary to correlate changes in EC_{50} values with loss of fungicide efficacy and reduced disease control.

Baseline sensitivity to the strobulurin (QoI) fungicide Headline was calculated using *C. beticola* isolates from our culture collection that were not previously exposed to Headline. Compared to this baseline, sensitivity of C. *beticola* to Headline has remained relatively stable from 2003-2009 with only an 8-10 fold decrease in sensitivity compared to the baseline (**Figure 6**). It should be emphasized that we have found isolates in the population with EC_{50} values >1.0 µg/ml (a 400 fold decrease in sensitivity) for Headline. There are numerous examples in many crops where resistance has developed to this class of fungicides. Because of the widespread application of Headline to sugar beets at the end of the season in our region, the application to many 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 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 and develop fungicide resistance management strategies with this goal as a high priority to insure effective management of *Cercospora beticola* for the long term.

SUMMARY

1. Resistance to Tin at 1.0 µg/ml has almost disappeared in our region, presumably 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 2009, 2% of the isolates were resistant to tin.

2. In 2009, only 14% of the samples were resistant to Topsin. It appears that the incidence of isolates resistant to Topsin has decreased dramatically since last tested in 2004. This reduction is somewhat surprising since resistance to benzimidazole fungicides does not revert to sensitive quickly. We do not know the reason for the reduction in resistance, but we do know that with other benzimidazole fungicides, resistance may quickly return in the population when application of fungicides in this class resume.

3. Sensitivity to Eminent remains relatively stable: the average EC_{50}values and the number of isolates with an EC_{50} > 1.0 µg/ml doubled from 2003-2009, which may indicate the potential for reduced sensitivity to develop. Sensitivity of two newer triazole fungicides, Proline and Inspire, has also remained stable from 2007-2009, and parallels sensitivity results for tetraconazole. There was some variability in sensitivity to Eminent among factory districts.

4. Sensitivity to Headline remains relatively stable, but there are rare isolates identified with a 400 fold 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 yet, but a few resistant isolates > 1 µg/ml have been found which has the potential for concern.

5. It appears that the fungicide resistance management plan that we are following is working since there have been no fungicide failures in our area due to fungicide resistance. However, disease pressure has been low, and higher disease pressure may change fungicide sensitivity patterns.

6. 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 (QoI) per season
- A good three spray program is triazole, tin, strobilurin (QoI)
- Using the high label rate of all fungicides
- 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
- Apply fungicides in a manner to insure maximum coverage; the fungicides used for Cercospora leaf spot control are protectants; better coverage results in better control. Fungicides must be in place before *C. beticola* inoculum arrives.

Figure 1. Sensitivity to Tin of *C. beticola* isolates collected in ND and MN from 1998 to 2009 at 1.0 µg/ml as measured by bulk spore germination.

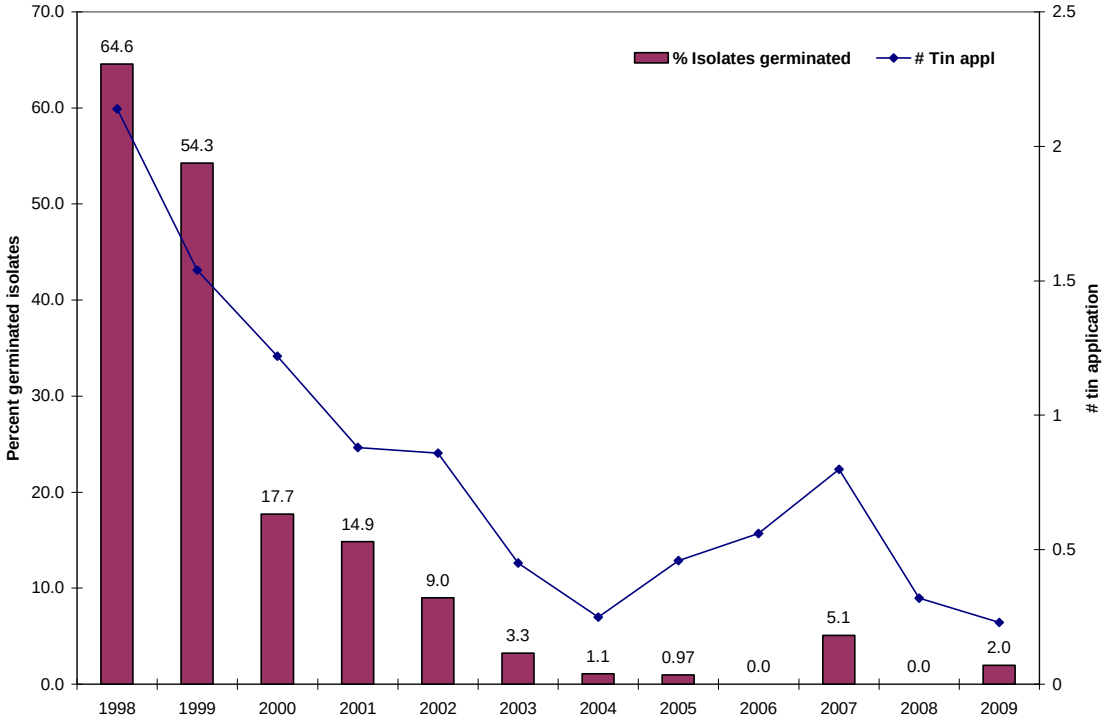

Figure 2. Percent germination of *Cercospora beticola* isolates collected in ND and MN from 2003 to 2009 on medium amended with Topsin at 5 µg/ml.

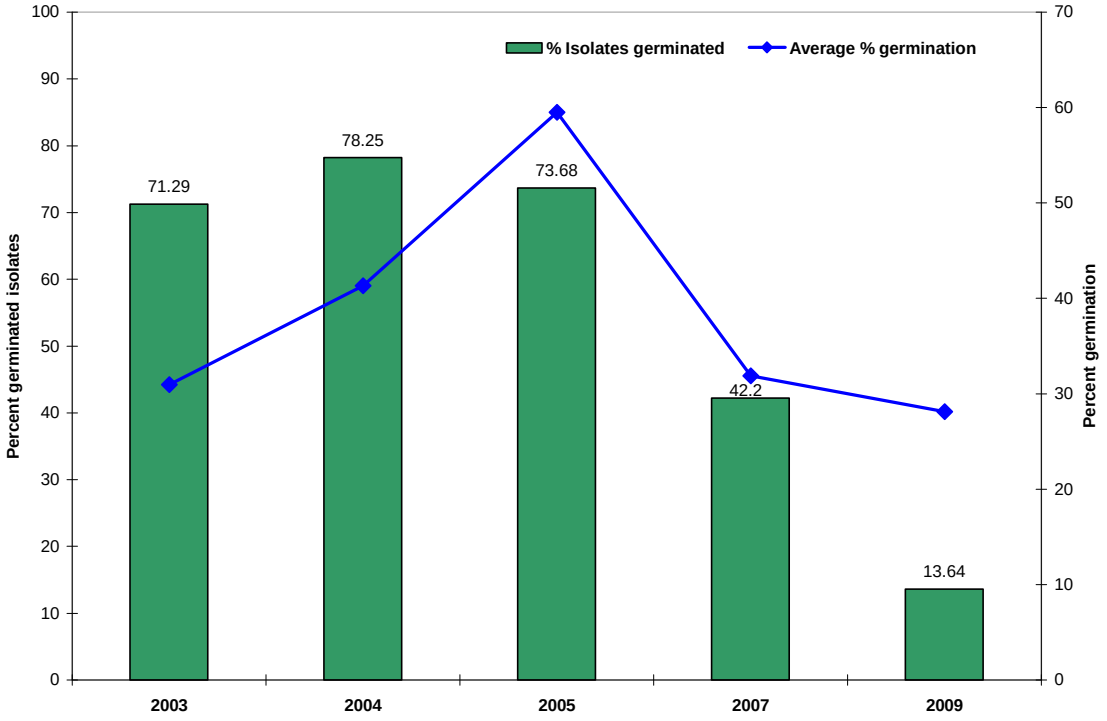

Figure 3. Average EC_{50} values of *C. beticola* isolates collected in ND and MN from 1997-2009 to tetraconazole.

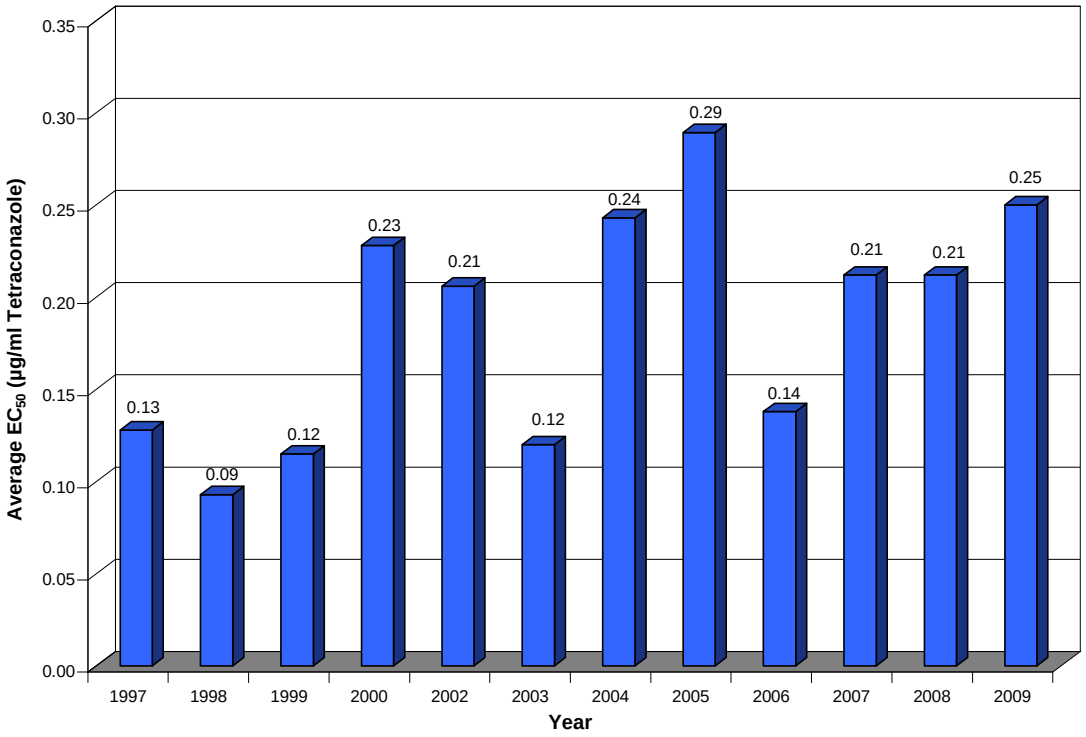

Figure 4. Average EC_{50} values of *C. beticola* isolates collected in 2007-2009 to three triazole fungicides.

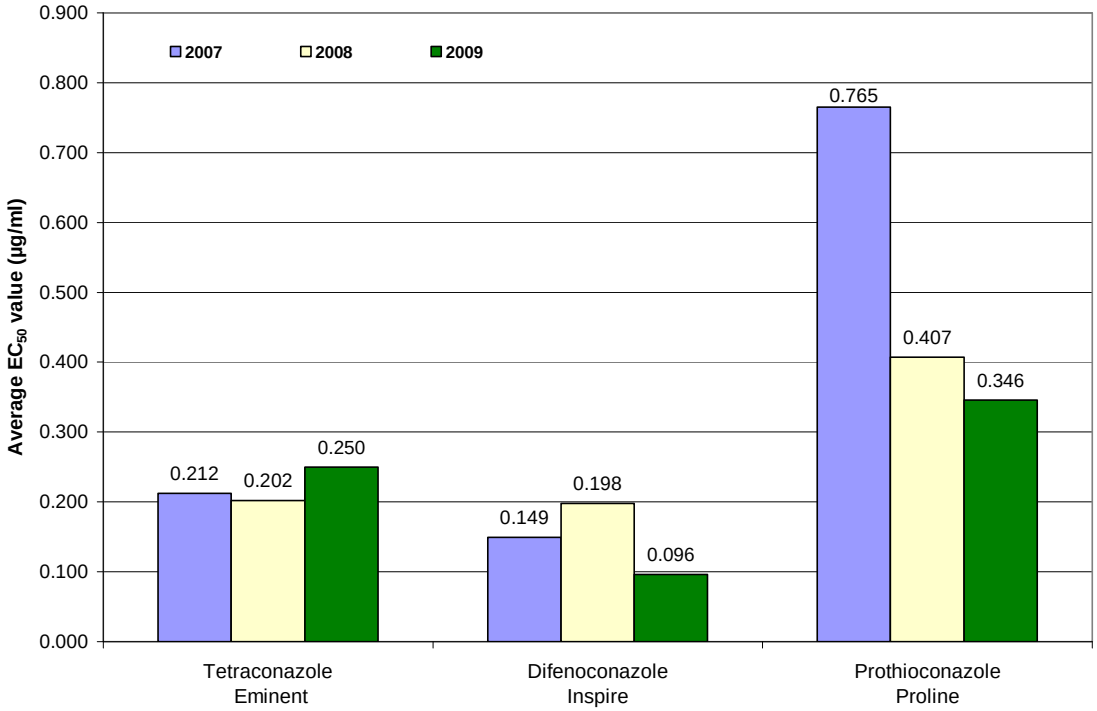

Figure 5. Sensitivity of *C. beticola* isolates to three triazole fungicides by factory district in 2009.

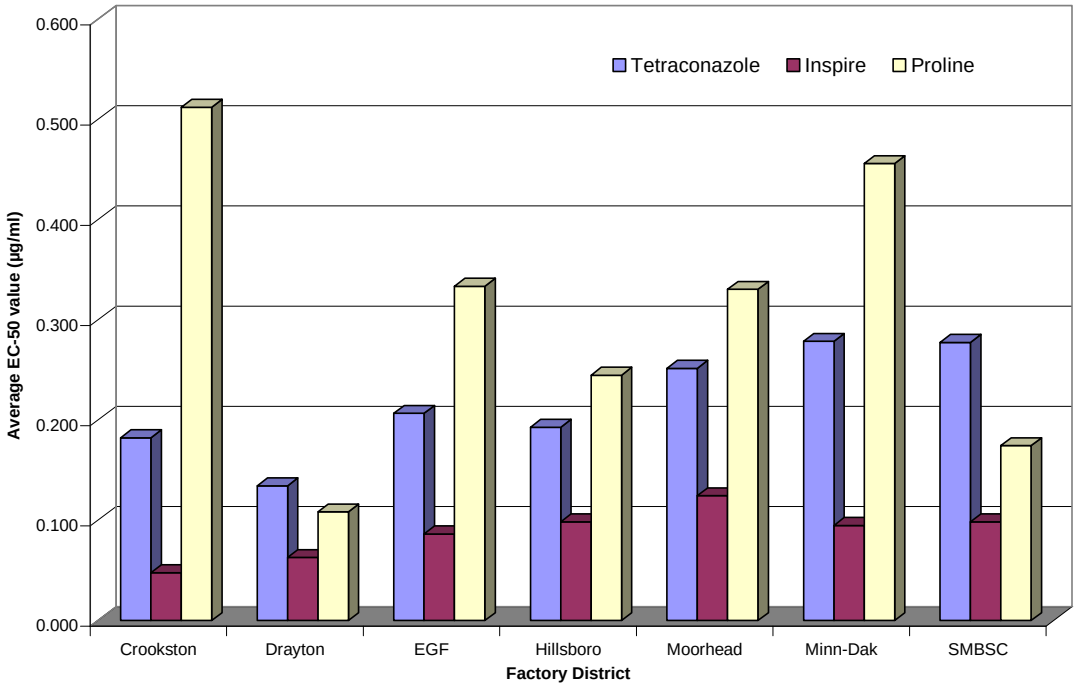

Figure 6. Average EC_{50} values of *C. beticola* isolates collected in ND and NM to pyraclostrobin (Headline) from 2003 to 2009.