SENSITIVITY OF *CERCOSPORA BETICOLA* TO FOLIAR FUNGICIDES IN 2011.

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Leaf spot, caused by the fungus *Cercospora beticola*, is an endemic disease of sugar beet 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 monitoring of field isolates of *C. beticola* to the commonly used fungicides in our area was conducted in the years 2003 - 2010. In 2011, sensitivity monitoring was conducted for tin, Topsin, Eminent, Inspire, and Headline.

OBJECTIVES

The 2011 objectives were:

1) Monitor 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) Monitor 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 two triazole (DMI) fungicides: Eminent (tetraconazole) and Inspire (difenoconazole).

4) Distribute results of sensitivity monitoring 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 2011, with financial support of the Sugarbeet Research and Extension Board of MN and ND, BASF Corporation, and Syngenta Crop Protection, 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, 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). This year for the first time, leaves with CLS were collected from both the upper canopy and the lower canopy. Isolates were collected from and tested separately from upper and lower leaves in order to determine of time of infection was associated with fungicide sensitivity. Ostensibly, lower leaves were infected earlier in the season than upper leaves.

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, 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 0.01 – 10.0 ppm. This if the first year we have tested for EC_{50} values between 1 and 10 ppm. A separate test was conducted for each triazole fungicide. 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 2011, disease pressure was generally low to moderate and Cercospora disease again developed late in the season. The majority of the CLS samples were delivered to our lab ate the end of the season in l September and October. Approximately 556 field samples representing all production areas and factory districts were tested for sensitivity to five fungicides in 2011. Additional samples (n=450) from fungicide trial plots of Dr. Mohamed Khan, NDSU 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%, in 2009 was 2.0%, and in 2010 was 1.4% (**Figure 1**). In 2011, the incidence of isolates resistant to tin at 1.0 ppm increased to 10.3% (**Figure 1**). The increase may be due to the increased use of tin plus Topsin in 2011 because of triazole resistance concerns. This increase is a beginning concern that deserves watching, as 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**). In 2011, the incidence of isolates resistant to Topsin at 5.0 ppm increased to 53.2% (**Figure 2**). It appears that incidence of isolates resistant to Topsin has increased dramatically since last tested in 2009. This rapid increase is not surprising, since resistance to benzimidazole fungicides does not revert to sensitive quickly, and resistance returns quickly when benzimidazole fungicides are used again.

Based on average EC_{50} values, overall resistance of *C. beticola* isolates to Eminent has doubled from 1998 to 2010 (**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, in 2009 was 0.25, and in 2010 was 0.26. In 2011, the average EC_{50} value increased to 1.40, almost an eight fold increase in resistance over the previous nine year average of 0.18. 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, and 19% in 2010. In 2011, 35.5% of the isolates tested had an EC_{50} value >1.0 ppm, some >10.0 ppm.

Based on average EC_{50} values, sensitivity to Inspire also increased The average EC_{50} values for Inspire were 0.15 in 2007, 0.20 in 2008 and 0.10 in 2009 and 0.17 in 2010 (**Figure 4**). In 2011, the average EC_{50} value increased to 0.48, almost a three fold increase in resistance over the previous four year average of 0.15. In 2009, the percent isolates in 2009 isolates with EC_{50} values >1.0 ppm to Inspire was 0.5%, in 2010 was 8.4%, and in 2011 was 9.5%, with a few >10 ppm.

Resistance to triazole fungicides increased in all factory districts (**Figures 5 and 6**). In general, there were no differences in EC50 values between lower leaves (early infection) and upper leaves (recent infection), but some differences were found in the Crookston and Drayton districts (**Figures 7 and 8**). Resistance, defined as EC_{50} >1, in the US correlates with reduced disease control in field and greenhouse trials we have conducted. The resistance to the triazole fungicides we see in US isolates of *C. beticola* is related to overexpression of Cyp51 enzyme, and not due to a specific genetic mutation. In companion studies we have conducted, higher levels of resistance to triazole fungicides are present in *C. beticola* isolates collected from Italy and France than found in the RRV production area. It will be critical to monitor resistance to triazole fungicides in the RRV region due to their widespread use and increased resistance in recent years. It may be prudent to pursue registration of fungicides with new modes of action and/or fungicide mixtures to help manage fungicide resistance

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 of 0.003 ppm, sensitivity of C. *beticola* to Headline has remained relatively stable from 2003-2009 with only a seven fold decrease in sensitivity. The average EC_{50} value of RRV isolates during 2003-2009 was 0.022 ppm, but in 2010 it was 0.174 and in 2011 0.082 (**Figure 9**). The percentage of isolates with EC50 values >1 ppm to Headline was 0.5 % in 2009, 2.3% in 2010 and 3.7% in 2011. In 2011, EC_{50} values >1.0 ppm ranged from 1.1 to 3.8 ppm. There has been a 40 fold increase in the EC50 value over the baseline EC50 value of 0.002 ppm prior to 2004, the first year Headline was used. In general, there were

higher EC_{50} values to Headline on lower leaves in most factory districts (**Figure 10**), but at the SMBSC district, higher EC_{50} values were found on upper leaves (**Figure 10**). In *C. beticola* isolates collected from Italy in 2010, 27% of the isolates had EC50 values <1 ppm ranging from 1.5-43.6 ppm. A specific genetic mutation was found in these isolates that correlated with Headline resistance. In 2011, there was widespread field resistance to Headline in Michigan. This resistance was correlated with high EC50 values, and resistant isolates had a specific mutation similar to that found in Italy. Isolates from the RRV with high EC50 values are currently being tested for this mutation. It will be critical to continue monitoring for resistance to Headline in the RRV production area, particularly because Headline is often the only fungicide used and is used annually even in the absence of disease.

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 2011, there was an increase in isolates resistant to tin, ostensibly due to an increase in tin application. In 2011, 10.3% of the isolates were resistant to tin.

2. 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. In the past two years, sensitivity to both triazole fungicides has increased, dramatically so for tertaxonazole.

3. The average EC_{50} value of RRV isolates during 2003-2009 was 0.022 ppm, but in 2010 it was 0.174 and in 2011 0.082. The percentage of isolates with EC50 values >1 ppm to Headline was 0.5 % in 2009, 2.3% in 2010 and 3.7% in 2011. In 2011, EC_{50} values >1.0 ppm ranged from 1.1 to 3.8 ppm. There has been a 40 fold increase in the EC50 value over the baseline EC50 value of 0.002 ppm prior to 2004, the first year Headline was used. It will be critical to continue monitoring for resistance to Headline in the RRV production area, particularly because Headline is often the only fungicide used and is used annually even in the absence of disease.

5. It appears that the fungicide resistance management plan that we are following has been working since there have been no fungicide failures in our area due to fungicide resistance. Our monitoring program has detected several shifts toward decreased sensitivity to all fungicides used for control.

6. Combinations of fungicides with different modes of actions may be necessary to prevent reduced sensitivity of *C. beticola* to currently registered fungicides. New fungicides with new modes of action should be tested for efficacy for registration.

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

- 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 2011 at 1.0 µg/ml as measured by bulk spore germination

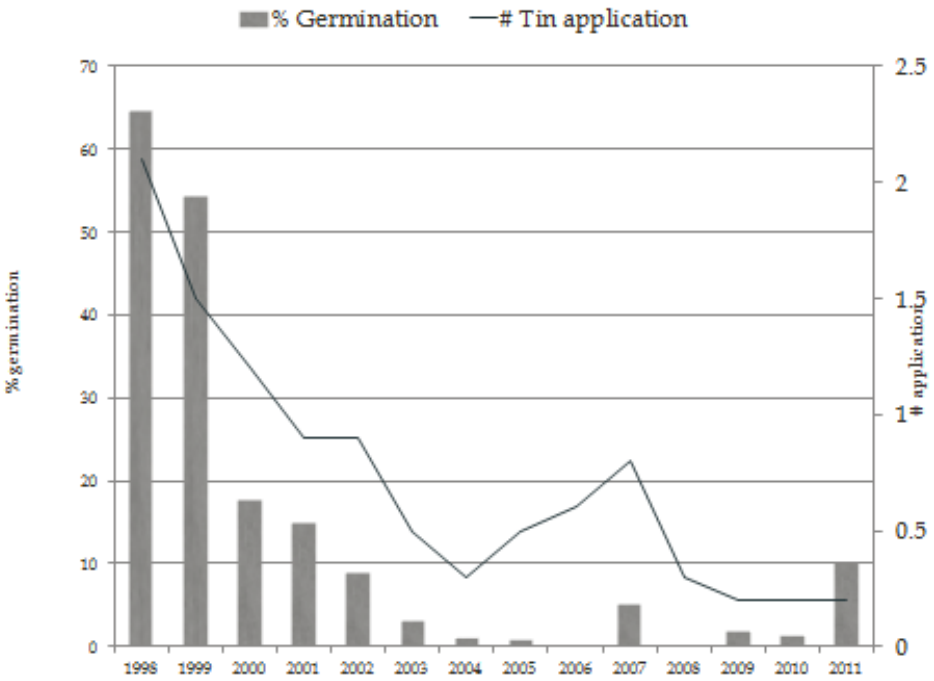

Figure 2. Percent germination of *Cercospora beticola* isolates collected in ND and MN from 2003 to 2011 on medium amended with Topsin at 5 µg/ml

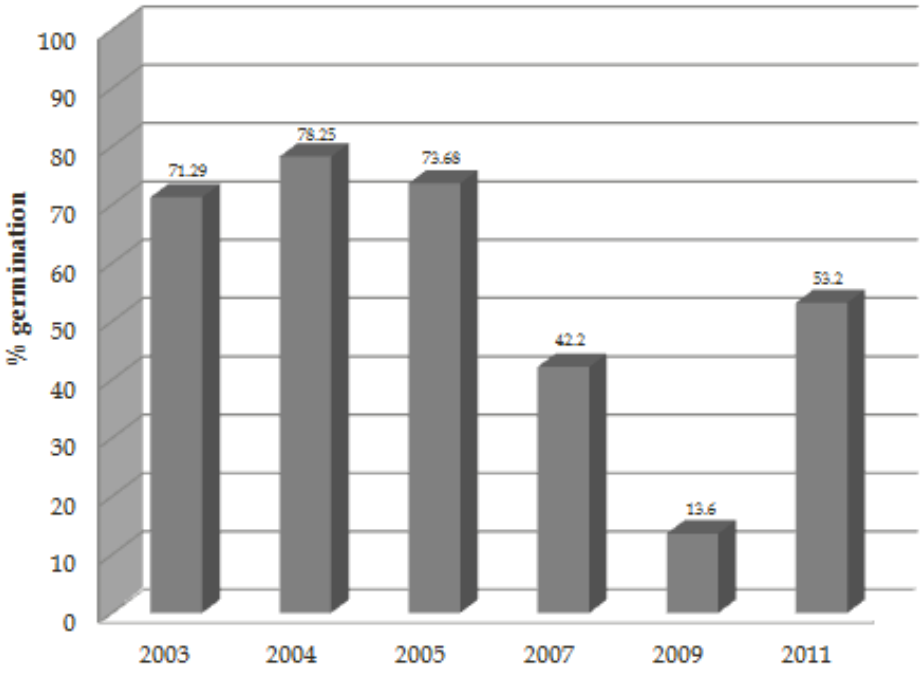

Figure 3. Average EC_{50} values of *C. beticola* isolates collected in ND and MN from 1997-2011 to Eminent

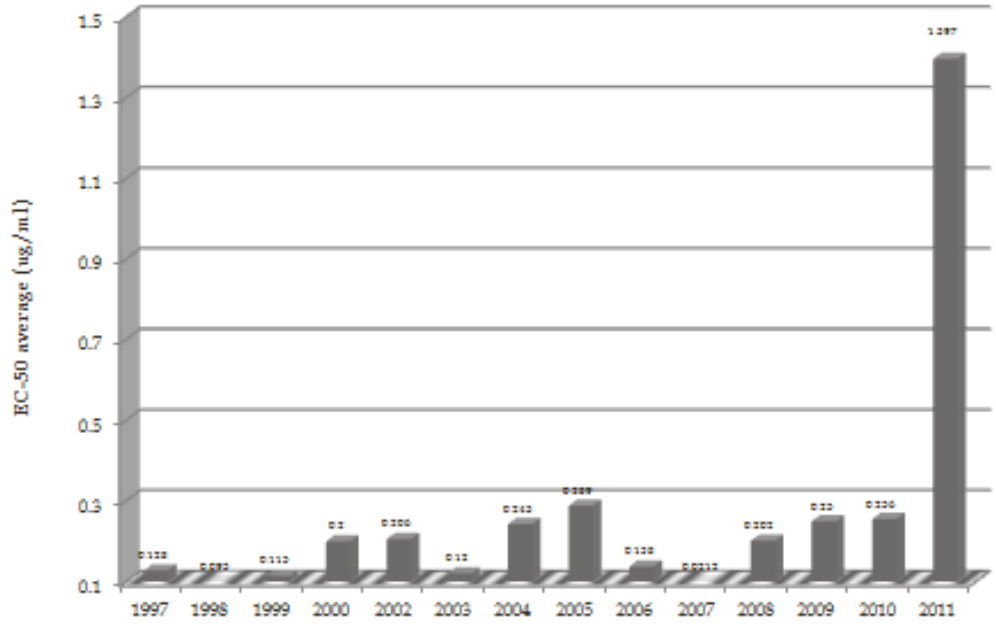

Figure 4. Average EC_{50} values of *C. beticola* isolates collected in ND and MN from 2007-2011 to Inspire

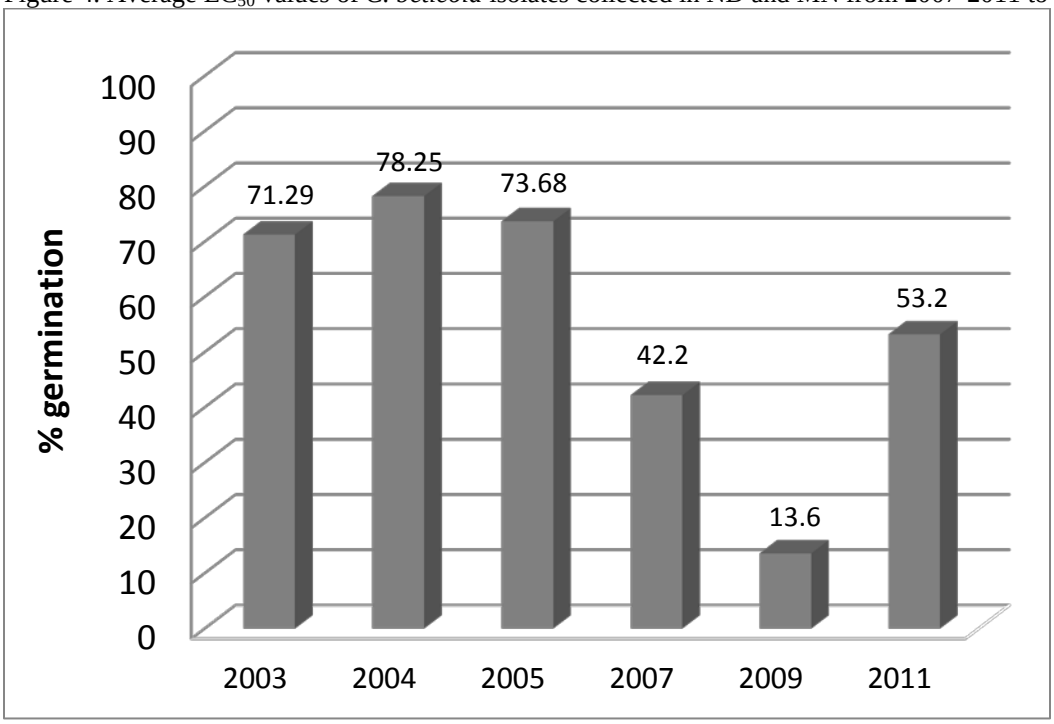

Figure 5. Average EC-50 values of *C. beticola* isolates collected in 2009-2011 to Eminent by factory district

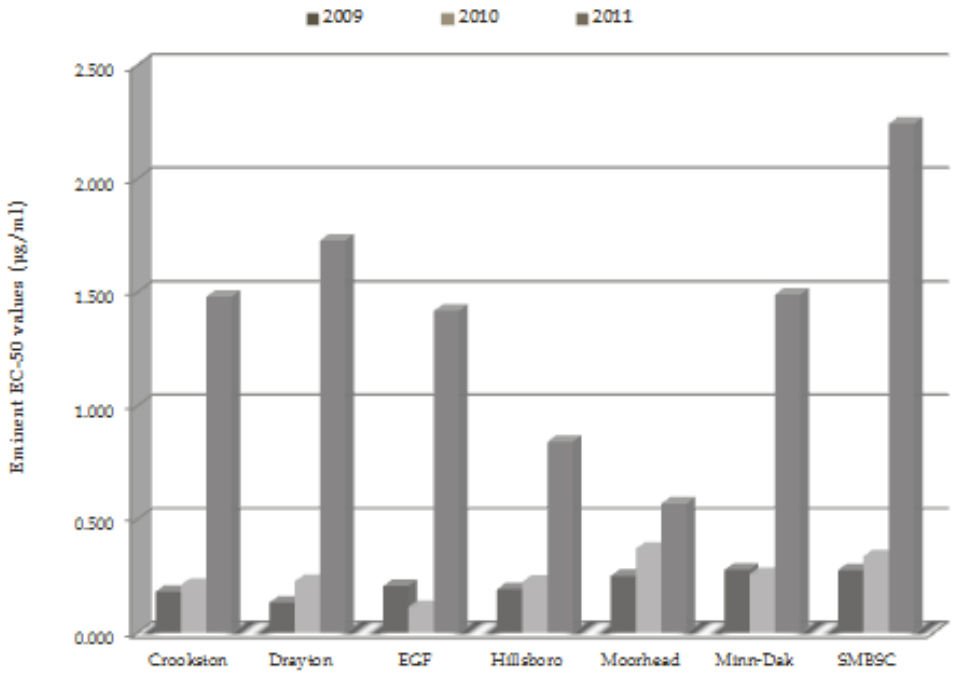

Figure 6. Average EC_{50} values of *C. beticola* isolates collected in 2009-2011 to Inspire by factory district.

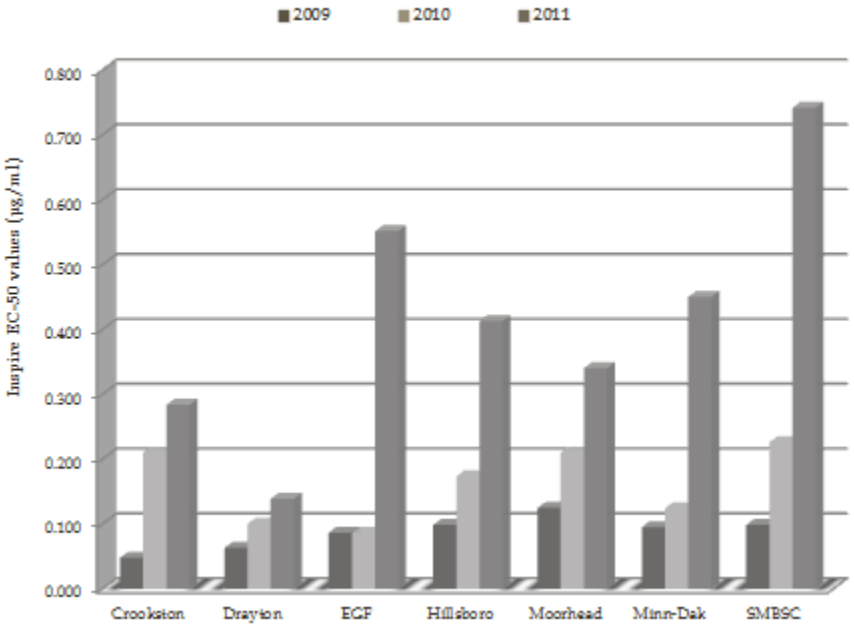

Figure 7. Average EC50 values of *C. beticola* isolates to Eminent from upper and lower canopy collected the same date

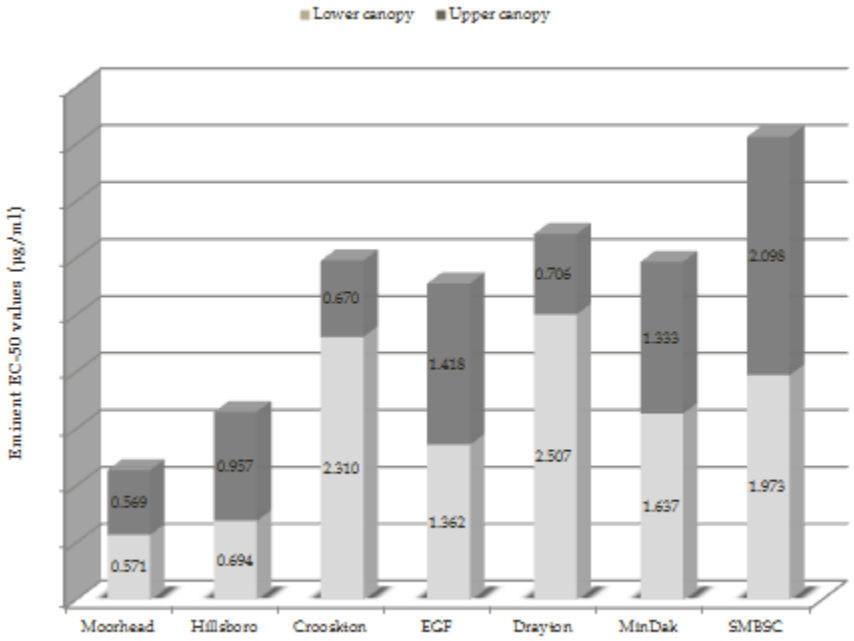

Figure 8. Average EC50 values of *C . beticola* isolates to Inspire from upper and lower canopy collected the same date

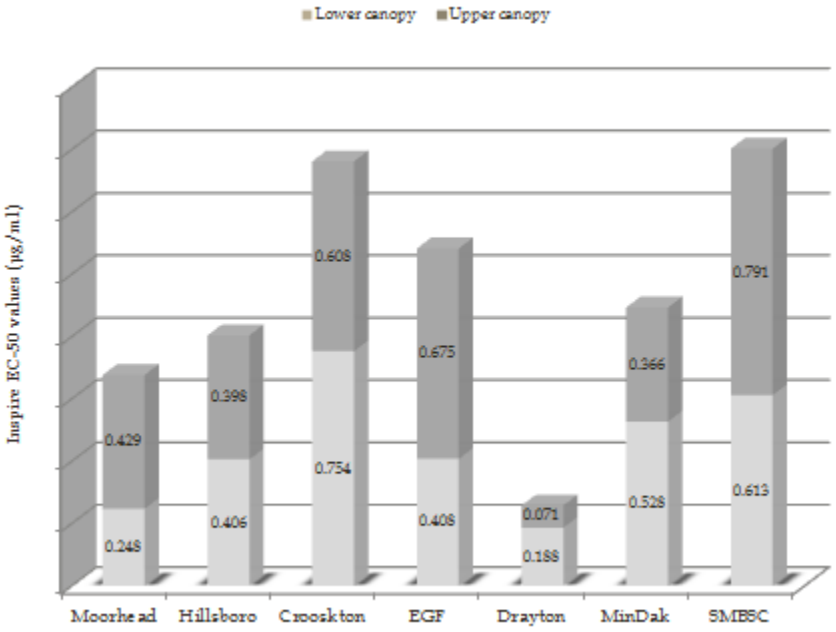

Figure 9. Average EC_{50} values of *C. beticola* isolates collected in ND and NM to Headline from 2003 to 2009

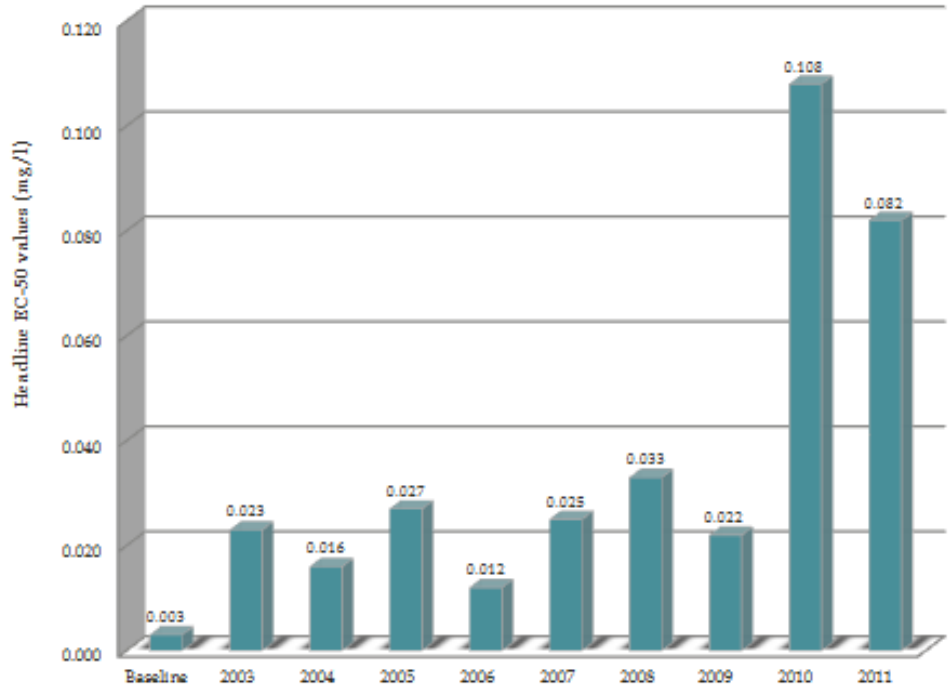

Figure 10. Average EC50 values of *C . beticola* isolates to Headline from upper and lower canopy collected the same date