SENSITIVITY OF *CERCOSPORA BETICOLA* TO FOLIAR FUNGICIDES IN 2019

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Leaf spot, caused by the fungus *Cercospora beticola*, is an endemic disease of sugarbeet produced in the Northern Great Plains area of North Dakota and Minnesota that reduces both yield and sucrose content. 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 multiple fungicide applications are necessary for disease management. Fungicides are used at high label rates and are alternated for best efficacy, but in recent years, mixtures are becoming more important. The most frequently used fungicides are Tin (fentin hydroxide), Topsin (thiophanate methyl), Eminent (tetraconazole), Proline (prothioconazole), Inspire (difenoconazole), and Headline (pyraclostrobin). Provysol (mefentrifluconazole) was registered for use on limited acreage in 2109. In 2019, most of the DMI and QoI fungicides were applied as mixtures with either mancozeb or copper and Topsin is usually applied as a tank mix with Tin.

Like many other fungi, *C. beticola* has the ability to become less sensitive (resistant) to the fungicides used to control them after repeated exposure, and increased disease losses can result. Because both *C. beticola* and the fungicides used for management have histories of fungicide resistance in our production areas and other production areas in the US, Europe and Chile, it is important to monitor our *C. beticola* population for changes in sensitivity to the fungicides in order to achieve maximum disease control. We have monitored fungicide sensitivity of field isolates of *C. beticola* collected from fields representing the sugarbeet production area of the Red River Valley region to the commonly used fungicides in our area annually since 2003. In 2098, extensive sensitivity monitoring was conducted for Tin, Topsin, Eminent, Inspire, Proline, Provysol and Headline.

OBJECTIVES

1) Monitor sensitivity of *Cercospora beticola* isolates to Tin (fentin hydroxide)

2) Monitor sensitivity of *Cercospora beticola* isolates to Topsin (thiophanate methyl) using PCR to detect the E198A mutationi

3) Monitor sensitivity of *Cercospora beticola* to four triazole (DMI) fungicides: Eminent (tetraconazole) and Inspire (difenoconazole) and Proline (prothioconazole) and Provysol (mefentrifluconazole)

4) Monitor *Cercospora beticola* isolates for the presence of the G143A mutation that confers resistance to Headline (pyraclostrobin) fungicide

5) Distribute results of sensitivity monitoring in a timely manner to the sugarbeet 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 2019, with financial support of the Sugarbeet Research and Extension Board of MN and ND, we tested 1230 *C. beticola* field isolates collected from throughout the sugarbeet production regions of ND and MN for sensitivity testing to Tin, Topsin, Eminent, Inspire, Proline, Provysol 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.

Sugarbeet leaves with Cercospora leaf spot (CLS) are collected from commercial sugarbeet 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 and delivered to our

lab for processing. From each field sample, *C. beticola* spores were collected from a minimum of five spots per leaf from five leaves and mixed to make a composite of approximately 2500 spores.

For Tin testing, a subsample of the spore composite was transferred to a Petri plate containing water agar amended with Tin at 1 ug/ml. Germination of 100 spores on the Tin amended water agar plates were counted 16 hours later and percent germination calculated. Germinated spores are considered resistant.

For Topsin testing, a PCR based molecular procedure was used to test for the presence of the E198A mutation in *C. beticola* that imparts resistance to Topsin. This is the second year the PCR test was used for testing for Topsin resistance and replaces the spore germination test.

For triazole fungicide sensitivity testing, a radial growth procedure is used. A single spore subculture from the spore composite is grown on water agar medium amended with serial ten-fold dilutions of each technical grade triazole fungicide from 0.01 – 100 ppm. A separate test is conducted for each triazole fungicide. After 15 days, inhibition of radial growth is measured, and compared to the growth of *C. beticola* on non-amended water agar medium. This data is 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. An RF (resistance factor) is calculated for each DMI fungicide by dividing the EC_{50} value by the baseline value so fungicides can be directly compared. Beginning in 2016, RF value calculations were increased to 10 ppm and in 2019 were increased to100 ppm to accommodate increased number of isolates with resistance to the DMI fungicides higher than 10 ppm.

For Headline resistance testing a PCR based molecular procedure was used to test for the presence of a specific mutation in *C. beticola* that imparts resistance to Headline. This procedure detects a specific mutation, G143A, which results in complete resistance to Headline. DNA is extracted from the remaining spore composite and tested by real-time PCR using primers specific for the G143A mutation. The test enables us to estimate the percentage of spores with the G143A mutation in each sample. The results are placed in five categories based on an estimate of the percentage of spores with the G143A mutation: S = no spores with G143A; S/r = <50 of the spores with G143A; S/R = equal number of spores with G143A; R/s >50% of the spores with G143A; and R = all spores with G143A**.**Each sample tested contains approximately 2500-5000 spores and the DNA from this spore pool will test for the G143A mutation from each spore. The PCR test is more sensitive and requires less interpretation than the previously used spore germination test. The PCR test will estimate the incidence of resistance in the population of spores tested, and give a better indication of Headline resistance in a field.

RESULTS AND DISCUSSION

CLS pressure was moderate in most locations in 2019, but cool temperatures and disastrous wet weather likely reduced disease pressure at the end of the season, especially in northern production areas. Disease pressure continued to be high in southern production areas. The majority of the CLS samples were delivered to our lab at the end of the season in late September and early October. Field samples (n=1097) representing all production areas and factory districts were tested for sensitivity to even fungicides: fentin hydroxide (Tin), thiophanate methyl (Topsin), tetraconazole (Eminent), difenoconazole (the most active part of Inspire), prothioconazole (Proline), mefentrifluconazole (Provysol) and pyraclostrobin (Headline). One additional DMI fungicide not registered in the US for CLS were tested for activity against *C. beticola*.

TIN. Tolerance (resistance) to Tin was first reported in 1994 at concentrations of 1-2 µg/ml. At these levels, disease control in the field is reduced. The incidence of fields with isolates resistant to Tin at 1.0 µg/ml increased between 1997 and 1999, but the incidence of fields with 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 incidence of fields with isolates resistant to Tin at 1.0 µg/ml was 64.6%, and declined to less than 10% from 2002 to 2010. From 2011 to 2014 there was an increase in the number of fields with resistance **(Figure 1),** and from 2015 to 2017, the incidence of fields

with isolates resistant to Tin increased from 38.5% to 97% (**Figure 1**). In 2018, the incidence of fields with isolates resistant to tin declined to 65.2% and declined again to 21.3% in 2019 (**Figure 1**). The severity of resistance, as expressed as percent germination of spores from fields with resistant isolates, ranged from 1 to 100%, with the average germination rate ranging from 16 to 28% during the five year period of 2013 to 2017 **(Figure 1)**. In 2018, spore germination declined to 15.5% and to 28.0 % in 2019. The incidence of fields with tin resistance declined dramatically in all factory districts except Moorhead and SMBSC (**Figure. 2**). The low severity of resistance (21.0 %) may be the reason that tin is still an effective fungicide for managing CLS despite widespread incidence of resistance to tin.

TOPSIN. Resistance to Topsin has been present in our area since 1999, and is also common and widespread in European Union production areas. Resistance has historically been >70% of the fields tested, but declined below that level in six years since 1999 (**Figure 3).** Topsin resistance, in sugarbeet and other crops, tends to decline when it is not used, but reappears quickly when it is again used in the field. Since 2013, the incidence of field with Topsin resistance was >70% (**Figure 3**). The incidence of fields with Topsin resistance in 201 increased to 88.6% and to 98.2% in 2019 (**Figure 3**). Data from 2018 and 2019 based on PCR testing for the E198A mutation. The severity of resistance, as expressed as percent germination of spores from fields with resistant isolates ranged from 1 to 100%, with the average germination rate of 25% in 2017. We were not able to test severity of resistance in 2018 and 2019 using the PCR test. Most applications of Topsin are as tank mixtures with Tin, which seems to be an effective management practice.

DMI (triazoles). Resistance as measured by RF values increased in 2019 for Inspire, Eminent and Provysol (**Figure 4**), but testing was extended to 100 ppm which may account for the increase. Isolates with RF values >100 ppm were detected for all four DMI fungicides (**Figure 5**), indicating increased resistance levels. Resistance was found in all factory districts, but there was some variability (**Figure 6**). RF values for Proline were low, but this was likely to using prothioconazole for testing instead of the active metabolic product desthioconazole for testing. Regardless, sensitivity to Proline was similar across all factory districts.

HEADLINE. Beginning in 2012, a PCR based molecular procedure was used to test for the presence of the G143A mutation in *C. beticola* using a composite spore sample containing approximately 2500-5000 spores. The presence of this mutation indicates absolute resistance to Headline. The G143A mutation was first detected in the RRV production area in 2012 and increased from 2013 to 2015. Resistance to Headline increased dramatically from 2016 to 2019 (**Figure 7**) and across all factory districts. In 2019, resistance to Headline continued to be at high levels similar to 2017 and 2018; resistance did not decline (**Figure 7**). (**Figure 7)**. Resistance was found at high levels in all factory districts, but resistance levels declined in the Minn-Dak factory district (**Figure 8**). This is a trend we hope continues, as we do not know if this mutation has the ability to revert to the sensitive wild type or not. We will continue to monitor 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. We do not know if there is a fitness penalty associated with the G143A mutation, but based on observation in MI and Italy, Austria and Serbia, where QoI resistance due to the G143A mutation is widespread. it appears that isolates with the G143A mutation are stable and can survive and increase in the population.

SUMMARY

1. Resistance to Tin at 1.0 µg/ml almost disappeared in our region from 2003-2010, but has increased since 2011, probably due to increased use. In 2019, the number of fields with tin resistance declined 36% and 65% in the past two years. The percentage of spores with resistance/field was stable at about 28 %. Efforts should continue to preserve this fungicide for Cls management. .

2. Resistance to Topsin continues to be present in our region at high levels. Topsin resistance was present in 98,2% of the isolates collected in 2019 using PCR testing for the E198A mutation that imparts resistance to Topsin. Topsin resistance remains in the population and Topsin is not an important resistance management partner. Topsin resistance was found in all factory districts.

3. This is where the action is. We now have four DMI fungicides available: Eminent, Proline, Inspire and Provysol. Resistance factors continue to increase for Eminent, Inspire and Provysol. Some isolates have RF levels >100 ppm, which is very high. Resistance to DMI fungicides is present in all factory districts with some differences. Proline had much lower RF values, this may be due to the testing procedure used. DMI fungicides should be applied a mancozeb or copper mixing partner. Copper inhibits spore germination. A PCR test has been developed to detect DMI resistance, this test may be validated for use in 2020.

4. The presence of isolates with the G143A mutation that results in resistance to Headline continues to be prevalent and widespread, as in 2017 and 2018. These findings preclude the effective use of Headline for CLS management in 2018. Headline is not recommended for Cls management but can be used for frost protection.

5. We recommend continuing disease control recommendations currently in place including fungicide rotation, using high label rate of fungicides, mixtures with mancozeb or copper, scouting at end of the season to decide the necessity of a late application, using fungicide resistance maps for fungicide selection, using a resistant variety, spray intervals of 14 days, and applying fungicides to insure maximum coverage. Improvements in fungicide coverage using proper spray nozzles and spray parameters such as timing, rate, interval and coverage should be implemented.

We urge the use of varieties with better Cls resistance, and these may be available in 2020.

Based on our lab observations, we recommend better cultural practices such as earlier fungicide application and destruction of initial inoculum at field edges to provide better disease control that will help with fungicide resistance management in Cls sugarbeet system.

Figure 1. Incidence and severity of tin resistance in *C. beticola* isolates collected from sugarbeet fields in ND and MN from 2003 to 2019

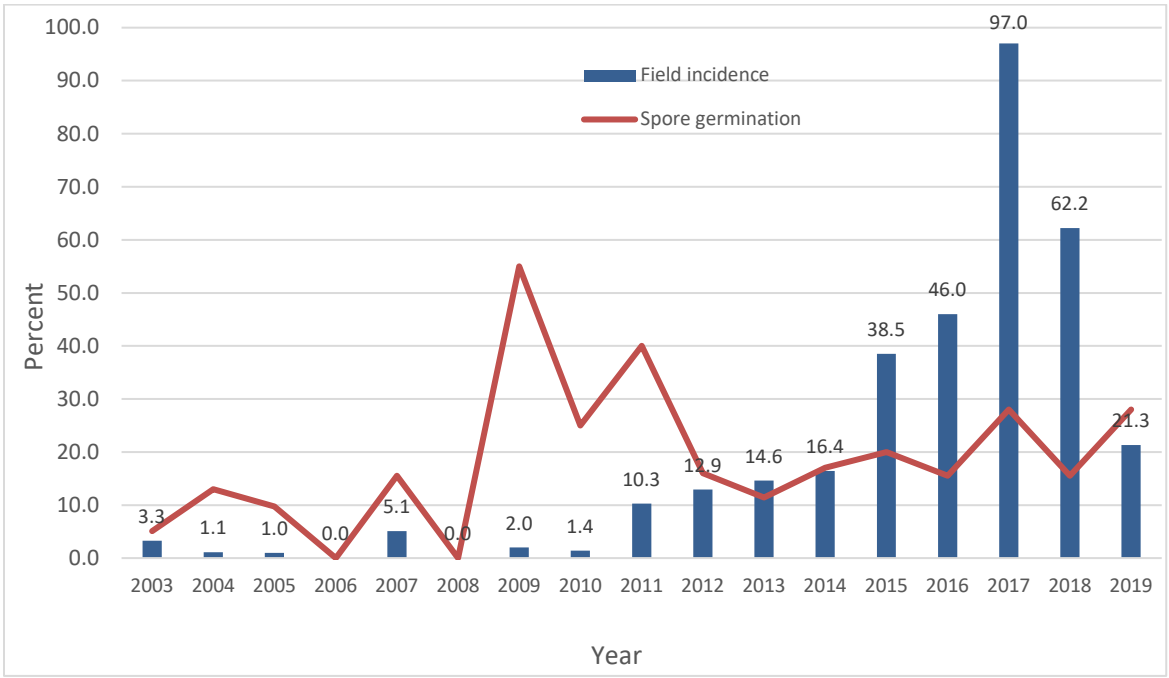

Figure 2. Incidence of fields with *C. beticola* isolates resistant to tin collected in ND and MN from 2015 to 2019 by factory dist

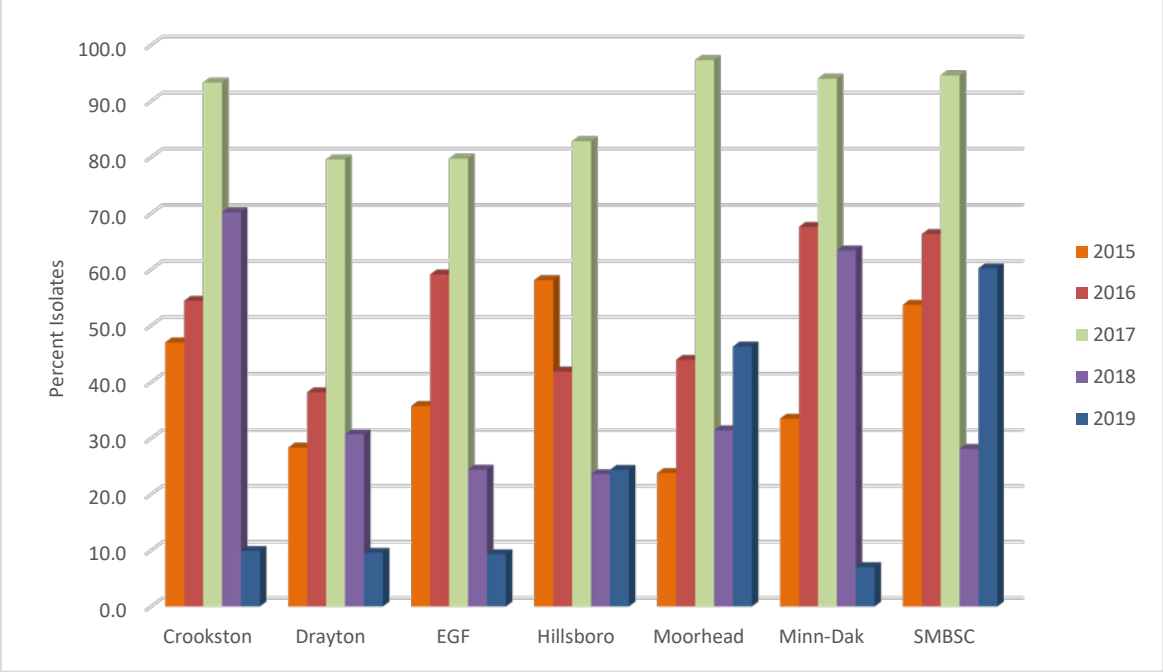

Figure 3. Percent of *Cercospora beticola* field isolates collected in ND and MN from 1999 to 2018 with growth on medium amended with Topsin at 5 µg/ml (* Data from 2018 and 2019 based on PCR testing for the E198A mutation)

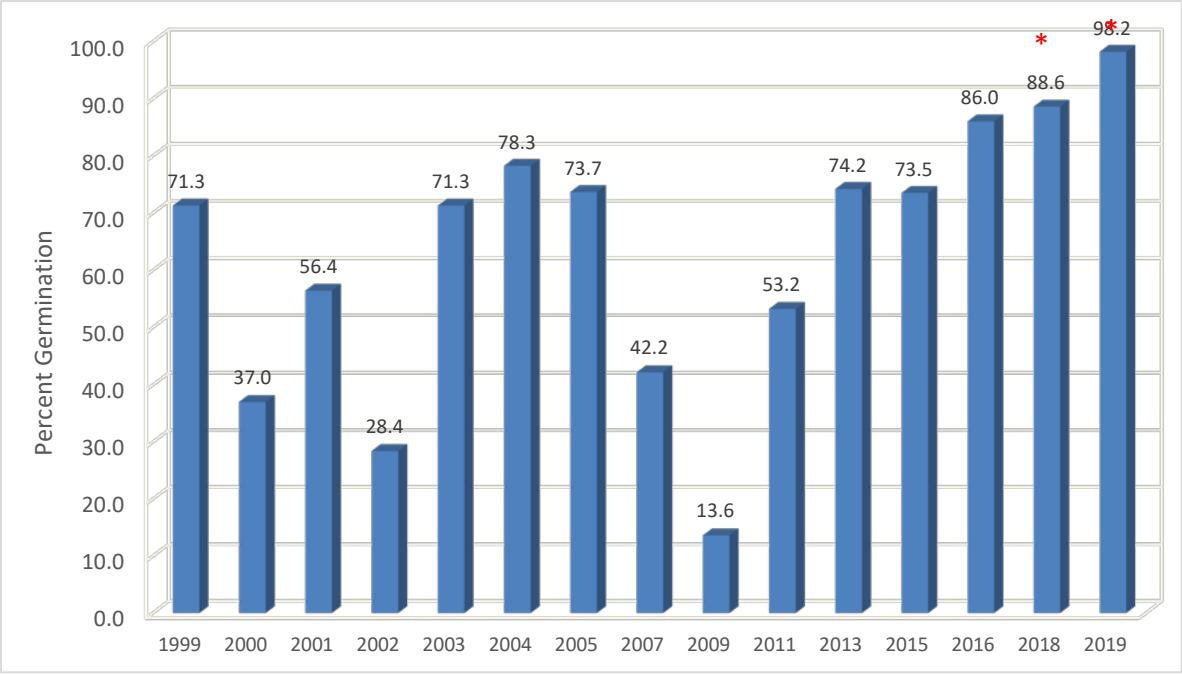

Figure 4. Resistance Factor of C. beticola isolates collected in ND and MN from 2017 to 2019 to Eminent, Inspire Proline and Provysol

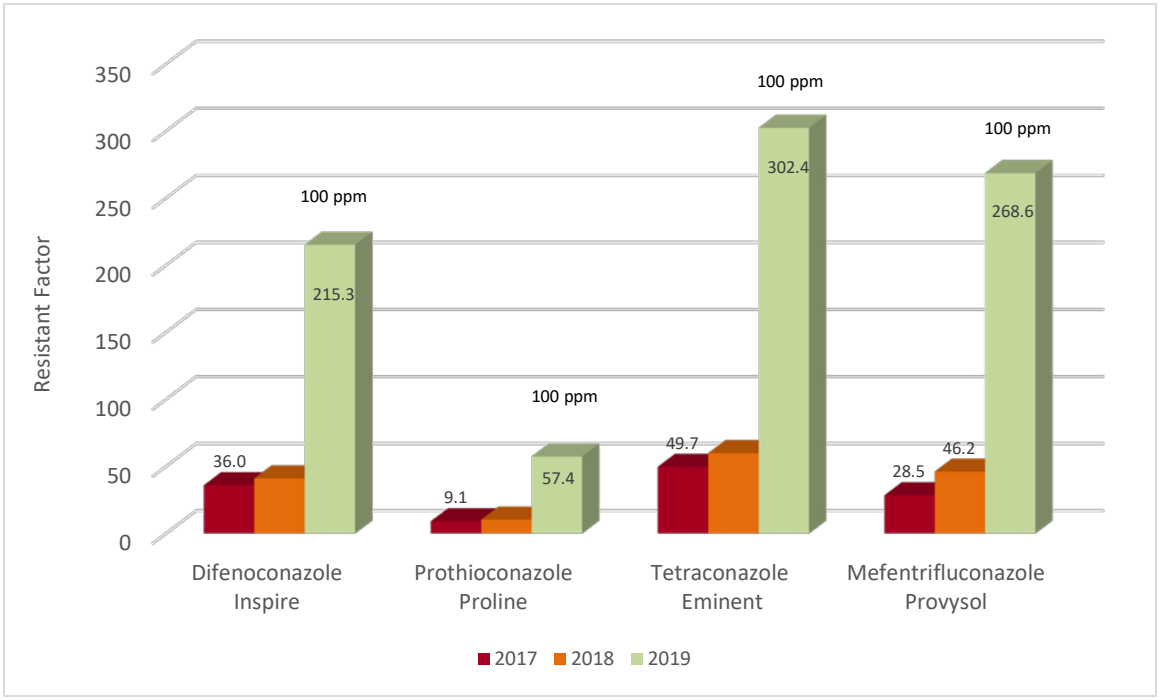

Figure 5. Distributin of sensitivity to Eminent, Inspire, Proline and Provysol of *C. beticola* isolates collected in 2019 as expressed by RF values

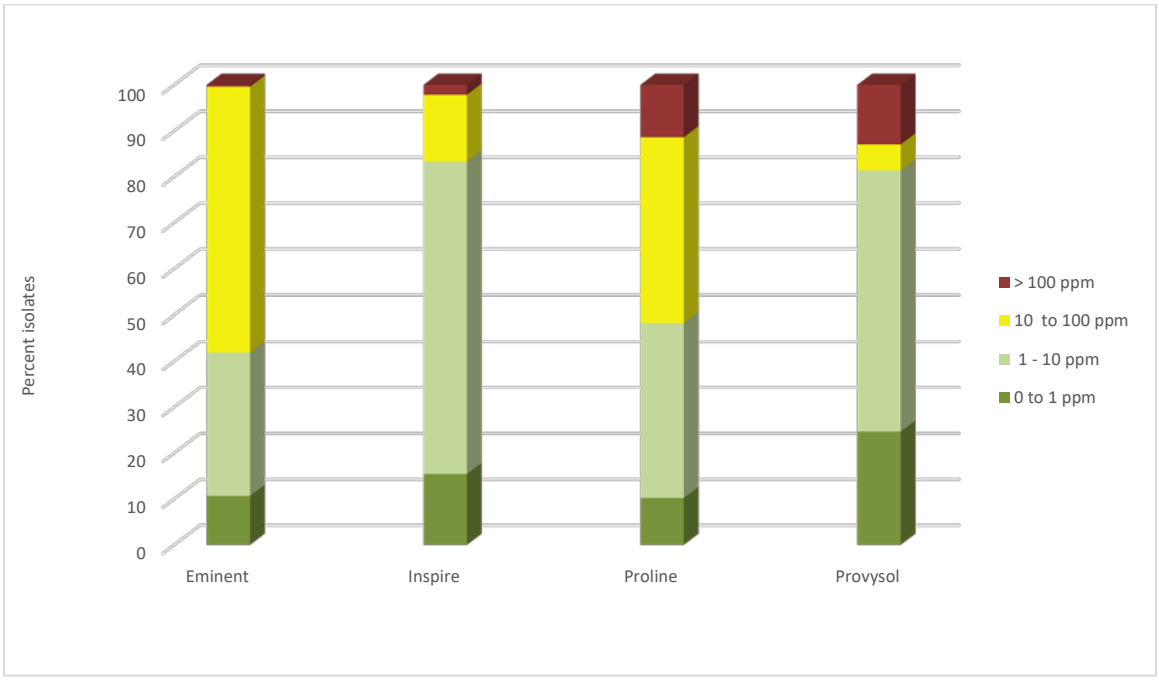

Figure 6. Sensitivity of *C. beticola* isolates collected in 2019 to Eminent, Inspire, Proline* and Provysol by factory district as expressed by RF values

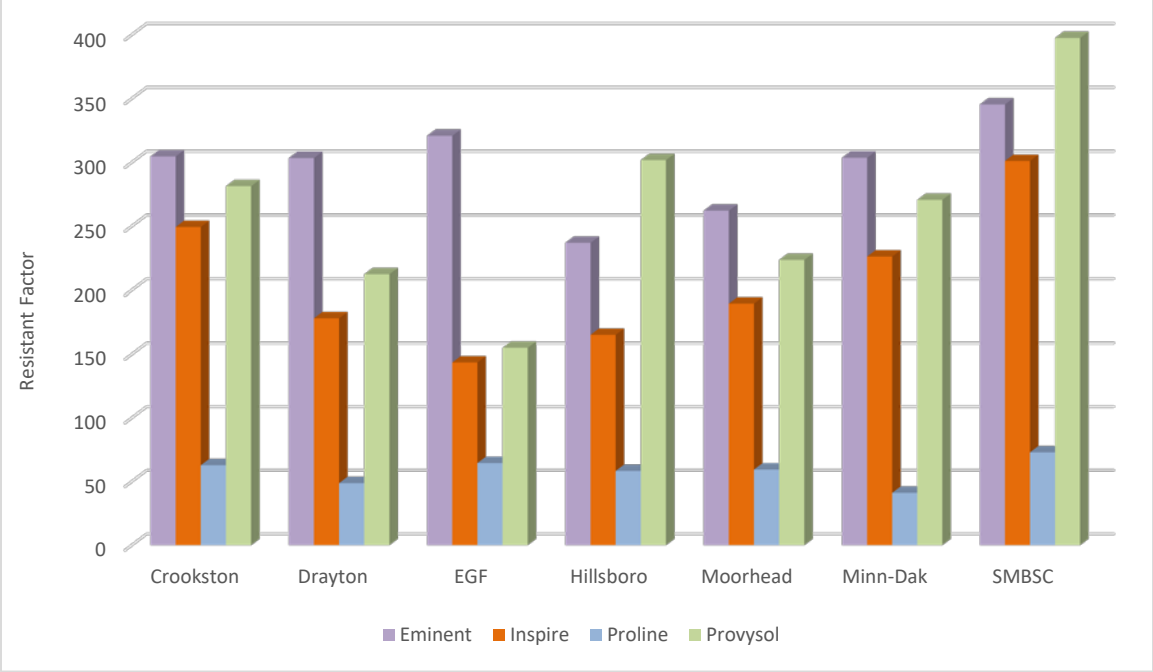

Figure 7. Sensitivity of *C. beticola* isolates collected in ND and MN to Headline from 2012 to 2019 as expressed by the percentage of spores with G143A mutation

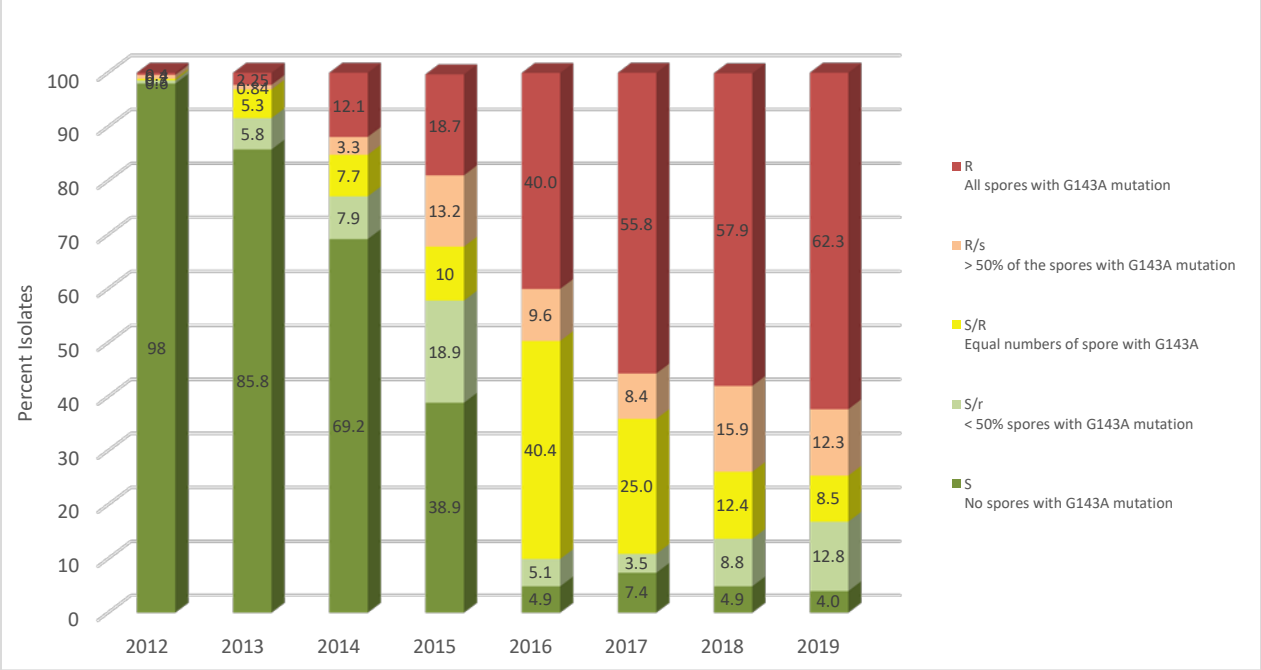

Figure 8. Sensitivity of *C. beticola* isolates collected in ND and MN in 2018 to Headline by factory district as measured by the percentage of spores with G143A mutation