EARLY SPORE DETECTION AND SENSITIVITY OF *CERCOSPORA BETICOLA* TO FOLIAR FUNGICIDES IN 2023

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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, cultural practices, resistant varieties and timely fungicide applications. *Cercospora* leaf spot usually appears in the last half of the growing season, but recent work has shown that spore production and infection happen much earlier necessitating earlier fungicide application. 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), Provysol (mefentrifluconazole) and Headline (pyraclostrobin). In 2023, most of the DMI fungicides were applied as mixtures with either mancozeb or copper.

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 2023, extensive sensitivity monitoring was conducted for Tin, Eminent, Inspire, Proline, Provysol and Headline.

OBJECTIVES

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

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

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

4) 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.

5) Monitor *Cercospra beticola* spore production in commercial sugar beet fields early in the growing season

METHODS AND MATERIALS

In 2023, with financial support of the Sugarbeet Research and Extension Board of MN and ND, we tested 673 *C. beticola* field isolates collected from throughout the sugarbeet production regions of ND and MN for sensitivity testing to Tin, 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 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.

For the third year in a row, we placed Spornado spore traps in six commercial sugar beet fields to monitor early detection of C. beticola spores from early May to early July.

It is interesting to note that a higher number of leaves (14%) with infection by small spore Alternaria species was found this year compared to previous years.

RESULTS AND DISCUSSION

CLS pressure was low in most locations in 2023 and many growers applied first fungicide application earlier than normal based on recommendations by cooperative agronomists. C. beticola spores were detected in spore traps in all field locations (two in ACSC, two in MinnDak and two in SMBSC) in early may and all of June.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=673) representing all production areas and factory districts were tested for sensitivity to six fungicides: fentin hydroxide (Tin), tetraconazole (Eminent), difenoconazole (the most active part of Inspire), prothioconazole (Proline), mefentrifluconazole (Provysol) and pyraclostrobin (Headline).

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 resistance to tin increased dramatically in 2020 (68.3%) and 2021 (98.9%) and 2022 (100%) and but declined dramatically in 2023 (31.5%) **(Figure 1).** The severity of resistance, as expressed as percent germination of spores from fields with resistant isolates, also increased dramatically in 2020 (40%) and 2021 (63%) and 2022 (65%) but declined dramatically in 2023 (18%). The incidence of fields with tin resistance decreased in all factory districts (**Figure 2**). These decreases are likely due to a fitness penalty present in resistant isolates that lowers survival rate from one year to the next.

DMI (triazoles). Resistance as measured by RF values (EC50/baseline EC50) in 2023 remained steady for Inspire, Proline and Provysol, but increased 32% for Eminent (**Figure 3**). Resistanace profiles of Inspire and Provysol remained about the same as in 2022 (**Figure 4**), but the resistance profiles for both Eminent and Proline increased at the highest resistance values (>100) **(Figure 4**) Interestingly, Eminent EC50 resistance values increased from zero in 2022 to 30% in 2023. Resistance profiles were relatively consistent across all factory districts, with some variability. The low RF values and high resistance profile for Proline are not a concern because these values are likely due to using technical grade prothioconazole for testing instead of the active metabolic product desthioconazole. We do not know if sugar beet converts prothioconazole to desthioconazole as other plants do, but we do know that resistance profile EC50 values of isolates is 15-fold higher to prothioconzaloe compared to desthioconazole. This is the subject of future research.

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 in field populations increased dramatically from 2016 to 2022, and continued in 2023 (**Figure 5)**. Resistance to Headline did not decline in 2023 (**Figure 5**). 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. It appears there is a fitness penalty associated with the G143A mutationbassed on reduced Headline resistance in isolates collected at the beginning of the season compared to higher resistance in isolates collected at the end of the season.

SUMMARY

1. The number fields with tin resistance increased in 2021 to 2022 to almost 100%, and but declined dramatically in 2023by 31.5%. reduced to about stabilized in 2022. The percentage of spores with resistance/field declined from 65% in 2021 and 2022 to 18% in 2023.

2. Resistance profiles of Inspire and Provysol remained about the same as in 2022, but the resistance profiles for both Eminent and Proline increased at the highest resistance values (>100). Eminent EC50 resistance values increased from zero in 2022 to 30% in 2023. Similar across all factory districts.

3. The presence of isolates in a population with the G143A mutation that results in resistance to Headline continued to be prevalent and widespread in 2023 as in past years.

4. 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.

5. We also recommend first fungicide application much earlier than previously recommended as we have detected *C. beticola* spores in commercial fields even prior to emergence. Since the fungicides used are all protectants, they need to be in place before spore arrive. Work is ongoing to adjust the forecasting model to include environmental factors affecting spore germination.

Figure 1. Incidence and severity of tin resistance in *C. beticola* isolates collected from sugarbeet fields in ND and MN from 1998 to 2023

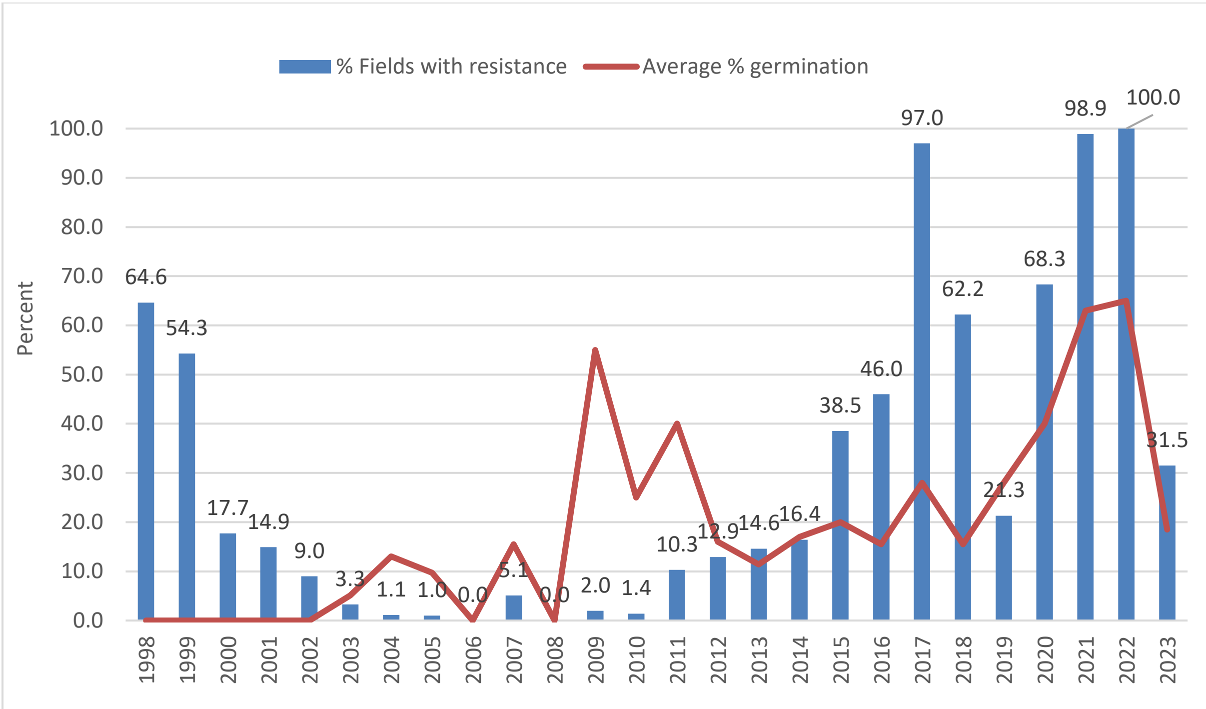

Figure 2. Incidence of fields with *C. beticola* isolates resistant to tin collected in ND and MN from 2020 to 2023 by factory district

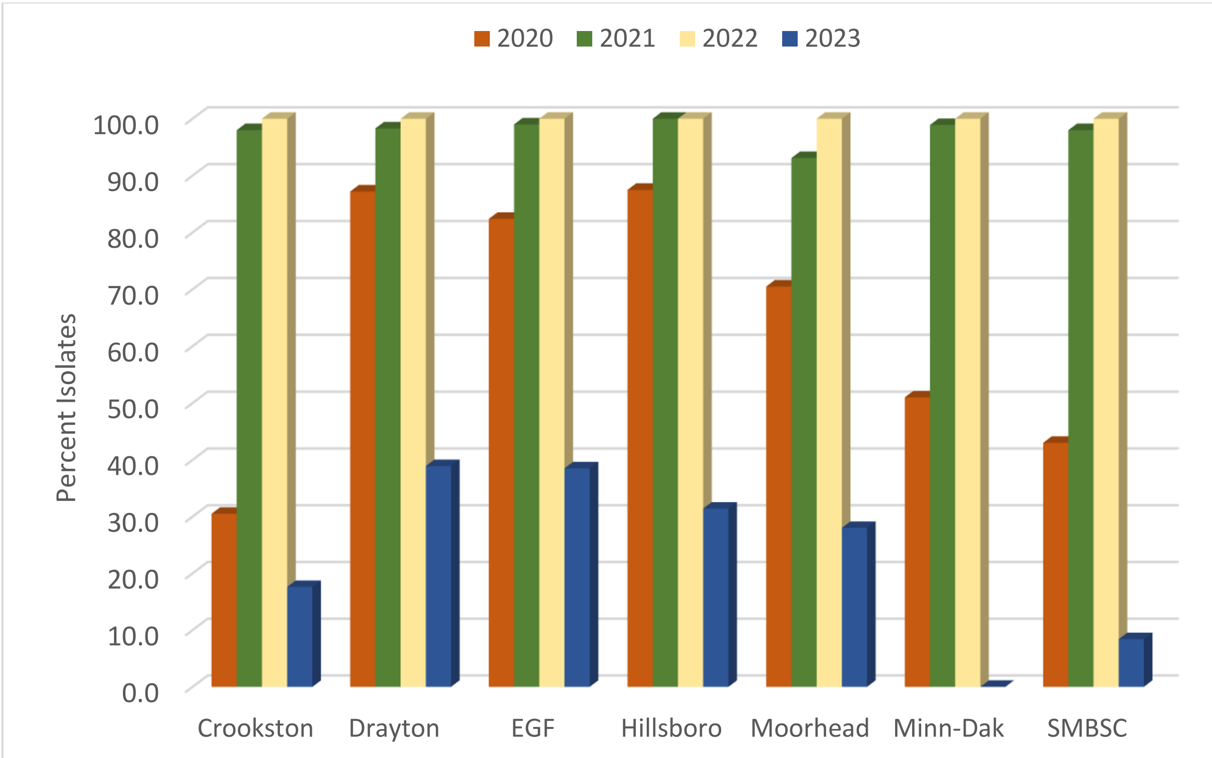

Figure 3. Resistance Factor of *C. beticola* isolates collected in ND and MN from 2019 to 2023 to Eminent, Inspire, Proline and Provysol

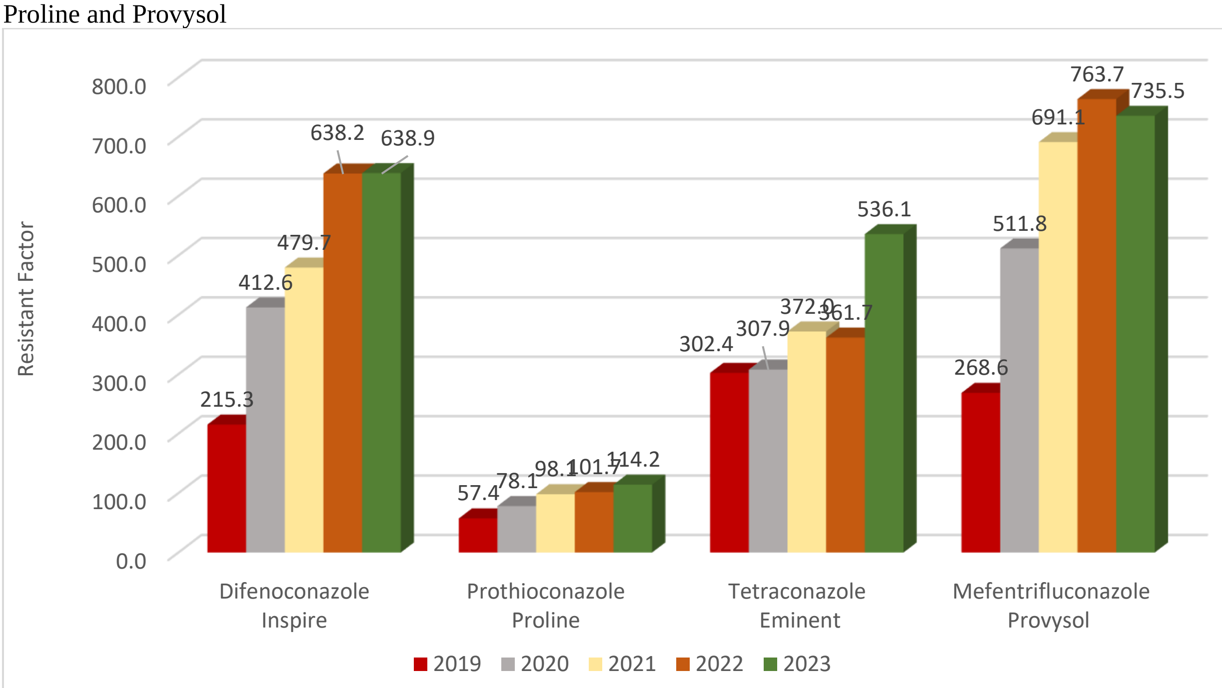

Figure 4. Sensitivity/resistance profile of Eminent, Inspire , Proline and Provysol as measured by EC50 values in 2022 and 2023

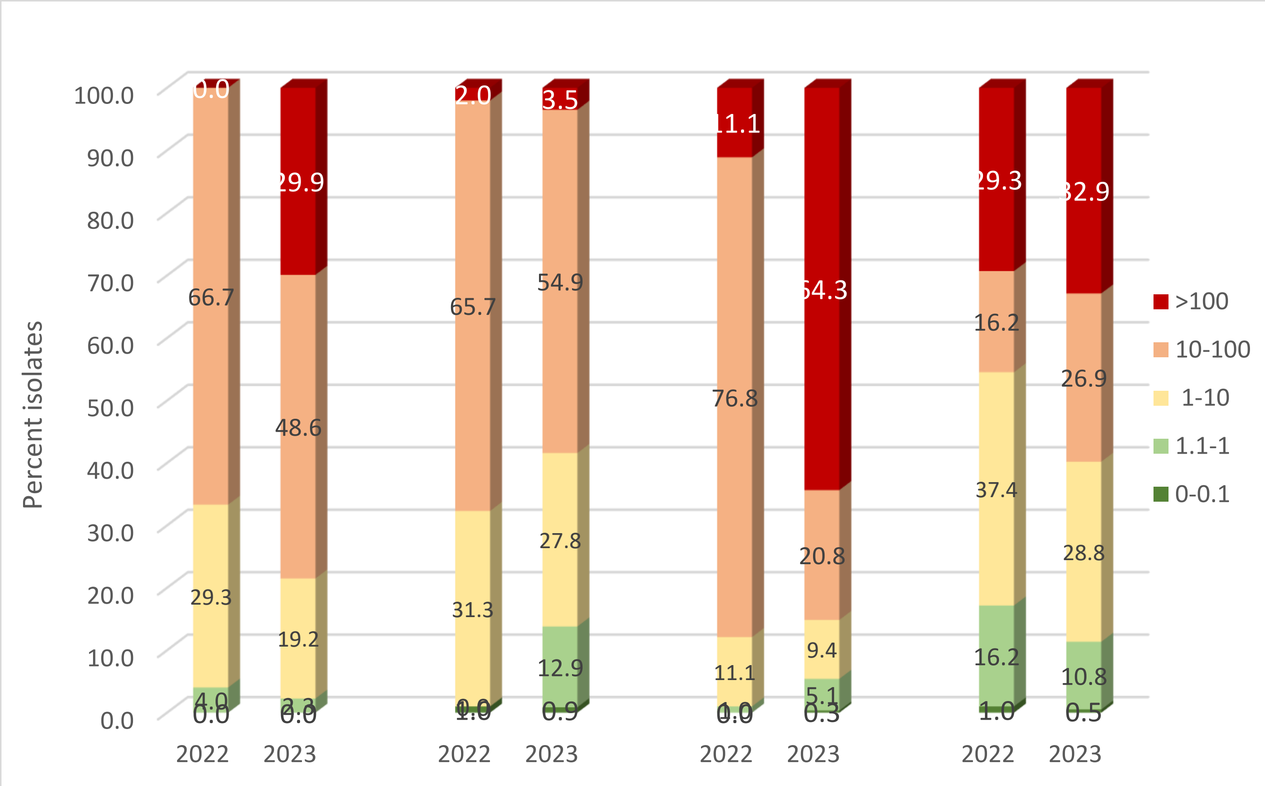

Figure 5. Profile of *C. beticola* isolates collected in ND and MN to Headline from 2012 to 2023 as expressed by the percentage of spores with G143A mutation