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Introduction

Cercospora leaf spot (CLS), caused by the fungus *Cercospora beticola*, is the most important foliar disease of sugarbeet throughout the world. It causes disease by reducing the photosynthetic area of leaves and producing a toxin that damages leaf cells. CLS has become endemic in the Red River Valley sugarbeet production area of eastern North Dakota and western Minnesota that accounts for about 60% of the total US crop, producing 13 million metric tons with yields of 42-28 mt/ha.

The disease is controlled by crop rotation, resistant sugarbeet varieties and timely fungicide applications. The disease usually appears in the last half of the growing season, and two to four fungicide applications are made during this time for disease control. The fungicides are applied singly, are used at highest label rates and are alternated during and between growing seasons. However mixtures of fungicides with different modes-of action are being tested and are becoming more common.

CLS is a polycyclic disease caused by repeating spore cycles, and the sexual stage of *C. beticola* is not known and is not important in the epidemiology of the disease. 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 several years. Because *C. beticola* has a history of resistance to the fungicides use to manage it, we developed and implemented a process to monitor *C. beticola* populations from the Red River Valley of North Dakota and Minnesota for changes in fungicide sensitivity that has been in place since 2000. In recent years, we have been testing populations from other states and some European countries as well.

Monitoring and Testing

Sugarbeet leaves with Cercospora leaf spot (CLS) are collected from commercial sugarbeet fields by agronomists from the three farmer-owned cooperatives that represent all production areas in ND and MN. From each field sample, *C. beticola* spores are collected from a minimum of five CLS lesions/leaf from each of five leaves from different plants and combined into a single composite of approximately 2500 spores in a small volume of water. This composite of spores is tested each year for sensitivity to the most commonly used fungicides, Tin (triphenyltin hydroxide), Eminent (tetraconazole), Inspire XT (difenoconazole plus propiconazole) and Headline (pyraclostrobin). Eminent and Inspire are DMI/triazole fungicides and Headline is a QoI fungicide. Sensitivity testing is conducted on an irregular basis for Topsin (a benzimadazole), Proline (DMI) and Gem (QoI). Technical grade active ingredient of each fungicide is used for testing, not the formulated commercial fungicide.

Sensitivity to tin is assessed by counting germination of 100 random spores on water agar amended with tin16 hours after plating and percent germination calculated. Spores that germinate are considered resistant. Germination on non-amended media was calculated for comparison. Resistance to tin was first reported in 1994, and at these levels, disease control in the field was reduced. The incidence of isolates with resistance to tin increased between 1997 and 1999, but has remained low since the introduction of additional fungicides for resistance management, including Eminent in 1999, Gem in 2002 and Headline in 2003, Inspire in 2008, and Proline in 2008. The registration of additional fungicides resulted in a decrease in the number of tin applications per season which in turn resulted in less selection pressure for triphenyltin hydroxide-resistant mutants of *C. beticola*. This suggests that tin sensitive isolates may be able to outcompete resistant isolates in the field, but more data is needed to substantiate this premise.

Resistance to the benzimidazole fungicide Topsin quickly became widespread in *C. beticola* in the 1980's in many sugar beet production areas of the US, including the Red River Valley. From 1998-2011, resistance to Topsin as measured by bulk spore germination at 5.0 ppm ranged from a high of 78.3% in 2004 to a low of 14.0% in 2009, and rebounded to 53.2% in 2011. It appears the even though the incidence of resistance to thiophanate methyl may fluctuate from year to year, resistance remains in the population and does not disappear. Topsin is almost always applied as a tank mix with tin.

For Eminent and Inspire, sensitivity change is measured by reduction of radial growth of cultures grown on water agar medium amended with serial ten-fold dilutions of each technical grade fungicide from 0.01 –10.0 ppm. After 15 days, inhibition of radial growth 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. Using this data, a resistance factor (RF) is calculated by dividing the EC_{50} values by the baseline EC_{50} value.

Based on RF values, overall resistance of *C. beticola* to Eminent was generally stable from 1998 to 2010 but increased dramatically in 2011-2012. The RF of field-collected isolates from 2002-2009 ranged from 1.0-2.3 µg ml-1. RF values increased to 12.7 in 2011, and to 34.4 in 2012, a 20 fold increase in resistance over the previous fourteen year average of 1.7. The number of *C. beticola* isolates with EC50 values >1.0 µg ml-1 has also been increasing. From 2002-2012 percentage of isolates with EC50 values of >1 ranged from 1.2% in 2002 to 65.4% in 2012.

Based on average EC_{50} values, sensitivity to difenoconazole has also increased. The RF values for Inspire from 2007-2010 ranged from 1.4-2.8. The average RF value increased to 10.7 in 2012, a five-fold increase in resistance over the previous four year average of 2.2. From 2009-2012 the percentage of isolates with EC_{50} values of >1 ranged from 0.5% in 2009 to 9.5% in 2012.

Based on field and greenhouse trials we have conducted, isolates with RF values >1.0 are considered resistant. The resistance to the DMI/triazole fungicides in isolates of *C. beticola* collected in our area is related to overexpression of Cyp51 enzyme, and not due to a specific mutation in the Cyp51 gene.

For the QoI fungicides, we have used a procedure that measures inhibition of spore germination to assess changes in fungicide senstivity. Spores are collected and transferred to water agar amended with ten-fold serial dilutions of Headline from 0.001 – 1.0 ppm. Germination of 100 spores is evaluated 16 hours after plating and percent germination calculated, and the reduction in germination is used to calculate EC_{50} values for each isolate. Using this data, a resistance factor (RF) is calculated by dividing the EC_{50} values by the baseline EC_{50} value. Compared to the baseline, the EC_{50} values of *C. beticola* to pyraclostrobin essentially did not increase from 2003-2009, but increased 4.8 fold in 2010 and 3.7 fold in 2011. We do not know the impact of this increase on fungicide efficacy.

A PCR based molecular procedure was used first in 2012 to detect a specific mutation in *C. beticola*, G143A, which imparts absolute resistance to Headline. For this test, DNA is extracted from spores collected from five spots from each of five leaves and tested by real time PCR using primers specific for the G143A mutation. Each sample tested contains approximately 2500 spores (100 each from five spots from five leaves for each field sampled) and the DNA pool for testing will test for the G143A mutation from each spore. The PCR test will estimate the incidence of resistance in the population of spores tested, and give a better indication of whether resistance is present in a particular field. The procedure can be completed in one day, compared to 14 days for the spore germination procedure. This will allow fields to be tested in advance in order to determine if Headline can be efficaciously applied. In 2012, only 1% of the 1127 field samples tested from our production area tested positive for the G143A 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. We do not know if there is a fitness penalty associated with the G143A mutation that enhances or reduces the competiveness or ability to overwinter of isolates with the G143A mutation. However, based on data from Michigan and Italy (our data), it appears that isolates of *C. beticola* that have the G143A mutation can persist and increase in the population.

Resistance management strategies

We advise growers, scouts and agronomists from the sugarbeet cooperatives to continue to use disease control recommendations for both disease and fungicide resistance management that have been developed over several years and are currently in place. These recommendations include:

— Fungicide rotations. These have been used most often in past years and continue to be necessary
    - Use only one triazole fungicide per season
    - Use only one QoI fungicide per season
    - A good three spray program is triazole, tin, QoI
    - Rotate both within and between seasons

— Fungicide combinations. These will be more common in future years
    - These include triazole + mancozeb, tin + Topsin, Headline + tin, triazole + Topsin etc.

— Use the high label rate of all fungicides; don't cut rates

— Scout at the end of the season to decide the necessity of a late application; CLS developed late in recent years due to long growing seasons

— Use some system to determine the first fungicide application. Can use web—based daily infection values available on the ND Agriculture Weather Network, row closure, first appearance of disease or the calendar

— Use fungicide resistance maps for fungicide selection. Avoid using a product in a region that has high levels of resistance

— Use spray intervals of 14 days using 20—25 gal/a by ground application and 5 gal/a by air application

— 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 need to be in place before *C. beticola* inoculum arrives.

— Grow a variety with resistance to CLS; our industry recommends planting of varieties with a KWS (Cercospora resistance) rating of <5.0
    - Resistance helps the fungicide work
    - Need to consider other variety traits

— Crop rotation of three years; our growers plant in quarter sections, so rotation is easy

Summary and challenges

Resistance to tin 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 2000. Registration of triphenyltin hydroxide is limited to the US, and it may be a challenge to maintain this registration in forthcoming years. The reduction in triphenyltin hydroxide resistance shows the importance of having numerous fungicides registered for decreasing fungicide resistance to reduce disease pressure in order to allow resistant isolates with a fitness penalty to naturally disappear from the field populations.

The rapid return of resistance to Topsin from 2009 to 2011 is not surprising since resistance to benzimidazole fungicides is stable and does not revert to sensitivity quickly, and resistance returns quickly when benzimidazole fungicides are again used. Topsin should only be used as a tank mix with tin where it shows good efficacy for disease control, and will limit resistance to this fungicide.

In the past two years, sensitivity to both Eminent and Inspire fungicides has increased, more so for Eminent. We know that Eminent resistance at >1 ppm results in disease control losses in greenhouse and field trials, and that disease control is better with Inspire than Eminent. The question remain whether resistance to one triazole results in resistance to all triazoles (is the resistance cross reactive?), or will the continued use of Inspire result in resistance levels in the near future we now see with Eminent. 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. The loss of triazole/DMI fungicides for managing CLS would be a devastating blow to our fungicide resistance management program.

After increases in EC_{50} values in 2010 and 2011, the G143A mutation was found for the first time in the Red River Valley that results in resistance to Headline was found in CLS samples collected from ND and MN. This is a stable and absolute mutation that has the potential to become severe and must be monitored for vigilantly. 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 for frost protection and increased sucrose content, although this has not been confirmed in all trials. We do not know if there is a fitness penalty associated with the G143A mutation that enhances or reduces the competiveness or ability to overwinter of isolates with the G143A mutation. However, based on data from MI and Italy, where this mutation became dominant in the population, it appears that isolates that have the G143A mutation can persist and increase in the population.

It appears that so far the fungicide resistance management plan that we are following has been effective since there have been no fungicide failures in our area due to fungicide resistance. However our monitoring program has detected several shifts toward decreased sensitivity to all fungicides used for control, and this needs continued monitoring.

Both alternation and combinations of fungicides with different modes of actions will be necessary in the future to prevent further reduction in sensitivity of *C. beticola* to currently registered fungicides. It may be prudent to pursue registration of fungicides with new modes of action and/or fungicide mixtures to help manage fungicide resistance, although this task will be difficult.

How does this apply to corn and soybean? The *Cercospora beticola* model could potentially serve as a model for resistance development and management of three *Cercospora* caused diseases of corn and soybean, *Cercospora sojina*, the cause of frogeye leafspot of soybean, *Cercospora kikuchii* the cause of leaf spot/purple seed stain of soybean and *Cercospora zeae-maydis*, the cause of gray leaf spot of corn,. Resistance of *C. sojina* to strobilurin fungicides was first reported in 2012 (Zhang et al), and according to Dr. Carl Bradley, University of Illinois plant pathologist, strobilurin fungicide-resistant strains of *C. sojina* have been found in at least eight Midwestern states. The QoI class of fungicides registered for soybeans includes azoxystrobin (Quadris, Quilt, Quilt Xcel), fluoxystrobin (Evito), pyraclostrobin (Headline) and trifloxystrobin (Stratego, Stratego YLD). The mutation causing QoI resistance is the G143A mutation which imparts absolute resistance and appears to be permanent. Resistance to QoI fungicides and to Topsin, and moderate increases in resistance to DMI fungicides, have been reported in the *C. kikuchii*/leaf spot/purple seed stain system in Louisiana (Price et al, LSU, 2013). Resistance to QoI fungicides may have applicability to the *C. zeae-maydis*/corn system but major fungicide sensitivity shifts have not been reported in this pathogen (personal communication, C. Bradley).

References

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