Monitoring Fungicide Sensitivity of *Cercospora beticola* of Sugar Beet for Disease Management Decisions

Cercospora leaf spot, caused by the fungus *Cercospora beticola* Sacc., is the most serious and important foliar disease of sugar beet (*Beta vulgaris* L.) wherever it is grown worldwide (21,24). *Cercospora* leaf spot first caused economic damage in North Dakota and Minnesota in 1980 (47), and the disease is now endemic. This is the largest production area for sugar beet in the United States, producing 5.5 to 6.0 million metric tons on approximately 300,000 ha, which is 56% of the sugar beet production in the United States (1). The sugar beet crop is produced by three grower-owned cooperatives, American Crystal Sugar Company, Minn-Dak Farmers Cooperative, and Southern Minnesota Beet Sugar Cooperative (Fig. 1). American Crystal Sugar Company, formerly American Beet Sugar Company, started operations at the East Grand Forks factory in 1926, became a grower-owned cooperative in 1973, and currently owns five factories located at Drayton, Hillsboro, East Grand Forks, Crookston, and Moorhead. Minn-Dak Farmers Cooperative and Southern Minnesota Beet Sugar Cooperative operate one factory each, and production commenced in 1974 and 1975, respectively (30). The sugar beet industry plays a vital role in the economic success of the region, as the 2003 sugar beet crop was estimated to generate total economic activity of US\$3 billion (2).

The sugar beet crop is grown under rain-fed conditions, and the favorable temperatures, frequent rains, and high humidity resulting in plentiful dew, provide conditions favorable for *Cercospora* leaf spot disease outbreaks. *Cercospora* leaf spot symptoms are circular spots about 3 to 4 mm in diameter with tan-gray centers and brown or purple borders which may cross the veins (Fig. 2). The spots can be scattered individually or coalesce and collectively cause a reduction in photosynthetic area, thereby reducing both tonnage and recoverable sucrose. In 1998, *C. beticola* caused estimated losses of \$45 million to American Crystal Sugar Company due to reduced yield and fungicide application costs (10,33). The disease is controlled by crop rotation, resistant varieties, and timely fungicide applications. *Cercospora* leaf spot usually appears in the second half of the growing season, and fungicide applications are made during this time for disease control depending on disease pressure and weather conditions. Since the major *Cercospora* leaf spot epidemic of 1998 (33), when the average number of fungicide applications per season was 3.74, the average number of applications per season has ranged from a high of 3.50 in 1999 to a low of 2.20

in 2008 (9,15). This is the story of how an industry has seen fungicide failure due to widespread resistance of *C. beticola* to fungicides, and has reacted to successfully develop a fungicide resistance management program for disease control and continued crop productivity.

Fungicides and Resistance Development

Processors provided sugar beet seeds to growers in North Dakota and Minnesota from 1926 to 1973. During that time, varieties had good resistance to *C. beticola* but generally had low yields and quality. Varieties with higher yields and better quality, but more susceptibility to *C. beticola*, were approved for use after growers bought the processing facilities and formed a cooperative in 1973. In the late 1970s, about 7 years after growers began using varieties that produced higher recoverable sucrose, *Cercospora* leaf spot became a problem because the varieties were more susceptible to *C. beticola* (47). Growers initially used three benzimidazole fungicides, benomyl (Benlate), thiabendazole (Mertect), and thiophanate-methyl (Topsin M) (Table 1), but *C. beticola* quickly developed resistance to these fungicides, resulting in an epidemic in 1981 (5,37). After the development of benzimidazole resistance in *C. beticola*, growers used primarily triphenyltin hydroxide (DuTer, Agsco TN, Triple Tin, Super Tin) fungicide for *Cercospora* leaf spot control (Table 1). In 1984, fungicide usage on sugar beet acres in North Dakota and Minnesota averaged 2.01 applications of triphenyltin hydroxide; mancozeb and benzimidazoles were applied once on less than 10% of the sugar beet acreage (16).

The widespread and repeated usage of triphenyltin hydroxide for control of *Cercospora* leaf spot continued without an effective alternating partner, eventually resulting in the development of resistance to triphenyltin hydroxide in the *C. beticola* population. Resistance to triphenyltin hydroxide was first reported (as tolerance) in 1994 in the Red River Valley (6,7) at levels between 1 and 2 µg/ml. At this level of resistance, there was a loss of disease control under these conditions. The incidence of resistance to triphenyltin hydroxide continued to increase, and by 1996, 60% of *C. beticola* isolates were resistant to triphenyltin hydroxide at 1.0 µg/ml (8). In 1998, favorable environmental conditions led to rapid development of *Cercospora* leaf spot and an epidemic since the pathogen was not controlled by the triphenyltin hydroxide fungicide applications, nor by azoxystrobin (Quadris) made available through an emergency exemption granted by the Environmental Protection Agency (EPA) (10,14). Resistance has also developed to fentin hydroxide and the benzimidazole fungicides in *C. beticola* populations in Europe (19,23,25).

In 1999, the EPA granted an emergency exemption for growers to use the nonregistered triazole fungicide, tetraconazole (Emi-

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ment), to control *Cercospora* leaf spot on sugar beet. Emergency exemptions continued through 2004, and tetraconazole was registered in 2005.

Two other triazoles, fenbuconazole (Enable) and propiconazole (Tilt), were registered for use in 2006, but due to lower efficacy, they are rarely used by growers (Table 1). Two additional triazoles, difenoconazole (Inspire SB) and prothioconazole (Proline), were registered for use in 2008 (Table 1). Two effective QoI fungicides became available for control of *Cercospora* leaf spot, trifloxystrobin (Gem) in 2002 and pyraclostrobin (Headline) in 2003 (Table 1). Currently, the most frequently applied fungicides are a QoI, pyraclostrobin (Headline), the triazoles prothioconazole (Proline), difenoconazole (Inspire SB), and tetraconazole (Eminent), and triphenyltin hydroxide (Super Tin, Agri Tin) (9).

Like many fungi, *C. beticola* has the ability to adapt and become less sensitive to the fungicides used to control it, especially if the fungicides are applied frequently over a period of time. In this paper, the terms *sensitive* and *resistant* are used to describe the reaction of *C. beticola* isolates to fungicides in accordance with previous recommendations (4); we use the definition of resistance as proposed by FRAC-UK to be “a change in the pathogen which results in decreased sensitivity to a fungicide, *nota bene*, slight changes are not generally obvious in the field and in most cases where disease control failure occurs, the change in sensitivity has been considerable”.

Sensitivity Testing

In order to maintain optimal disease control by fungicides, it is important to monitor the pathogen population for sensitivity and changes in sensitivity to these fungicides. To us, monitoring for fungicide resistance means “testing of field populations of target pathogens for their degree of sensitivity to one or more fungicides” (4). Because there has been a loss of *Cercospora* leaf spot control due to fungicide resistance in previous years, we utilized our culture collection of *C. beticola* isolates from 1997 to 2000 to establish baseline sensitivities to some fungicides before they became registered and to evaluate shifts in sensitivity to triphenyltin hydroxide and thiophanate-methyl from earlier periods. Fungicide sensitivity testing of field isolates of *C. beticola* to the commonly used fungicides in our area were conducted annually from 2003 in our laboratory. Previously, testing was done at the USDA, Agricultural Research Service Northern Crop Science Laboratory, Fargo, ND.

For resistance testing, sugar beet leaves with *Cercospora* leaf spot symptoms are collected from commercial fields by agronomists from American Crystal Sugar Company, Minn-Dak Farmers

Cooperative, and Southern Minnesota Beet Sugar Cooperative representing all production areas in North Dakota and Minnesota. Leaves are delivered and processed within 1 to 2 days to ensure viability of spores. From each field sample of *Cercospora* leaf spot, a composite sample of *C. beticola* spores is collected from a minimum of five spots per leaf from five leaves. The spores are collected by applying 100 µl of distilled water to each spot and flushing several times to dislodge the spores. The resulting suspensions (from 25 spots) are combined, and a 200-µl composite sample of the spore suspension is transferred to each of three petri plates containing water agar amended with triphenyltin hydroxide at 1 µg/ml, thiophanate-methyl at 5 µg/ml, or nonamended (water agar alone).

For triphenyltin hydroxide and thiophanate-methyl, sensitivity is monitored by calculating the incidence of spore germination in the presence of a discriminate dosage of 1.0 µg/ml of triphenyltin hydroxide or 5.0 µg/ml of thiophanate-methyl. These concentrations were selected based on previous work (6) that showed a loss of disease control in the field at these concentrations. For each field sample, germination of 100 spores viewed at random on water agar amended with triphenyltin hydroxide at 1 µg/ml or thiophanate-

Table 1. Chronology of fungicide usage for management of *Cercospora* leaf spot in North Dakota and Minnesota

Fungicide	Year of first use
Benomyl (Benlate)	1979
Thiabendazole (Mertect)	1979
Thiophanate-methyl (Topsin-M)	1979
Triphenyltin hydroxide (DuTer)	1979
Triphenyltin hydroxide (Agso)	1979
Triphenyltin hydroxide (Triple Tin)	1979
Triphenyltin hydroxide (Super Tin)	1979
Manganese ethylene-1,2-bisdithiocarbamate (Maneb)	1979
Dithiocarbamate (Mancozeb)	1979
Azoxystrobin (Quadris)	1998
Triphenyltin hydroxide (Agri Tin)	1999
Tetraconazole (Eminent)	1999
Trifloxystrobin (Gem)	2002
Pyraclostrobin (Headline)	2003
Fenbuconazole (Enable)	2006
Propiconazole (Tilt)	2006
Difenoconazole (Inspire)	2008
Prothioconazole (Proline)	2008

Fig. 1. Sugar beet cooperatives and production areas in North Dakota and Minnesota.

methyl at 5 µg/ml was counted 16 h after plating, and percent germination was calculated. Germinated spores are considered resistant, and it is assumed that each spore constitutes an isolate.

For triazole fungicide sensitivity testing, a standard inhibition of radial growth procedure across a range of fungicide concentrations modified for *C. beticola* was used to monitor changes (27). A single *C. beticola* spore subculture from the original nonamended water agar plate was grown on water agar medium amended with serial 10-fold dilutions of technical grade triazole fungicide active ingredient from 0.001 to 1.0 µg/ml. This type of procedure is necessary to detect slight changes in sensitivity of a fungus to a sterol inhibiting fungicide, since resistance to this class of chemical is inherited quantitatively (13,18,34). Separate tests were conducted for each triazole fungicide. After 15 days, radial growth was measured and compared to growth on nonamended water agar. These data were used to calculate an EC₅₀ value for each isolate; EC₅₀ is the concentration of fungicide that reduces growth of *C. beticola* by 50% compared to the growth on nonamended media.

For the QoI fungicides, we use a procedure that measures inhibition of spore germination. A subculture from the original nonamended water agar plate was grown on modified V8 medium and induced to sporulate abundantly (*unpublished*). *C. beticola* spores were collected and transferred to water agar amended with serial 10-fold dilutions of technical grade fungicide active ingredient from 0.001 to 1.0 µg/ml containing salicylhydroxamic acid at a concentration of 100 µg/ml. We knew from tests we conducted (*unpublished*) that *C. beticola* spores reach >80% germination in about 16 h, with some variability depending on isolate. Consequently, germination of 100 spores viewed at random was done 16 h after plating. Germination on amended media was compared to the germination on nonamended water agar medium and a percent germination calculated from which an EC₅₀ can be calculated for each isolate.

Fungicide Sensitivity Results and Changes

Samples are generally delivered to our laboratory for testing from early August through late October, but the majority (87%) of

Fig. 2. Foliar symptoms of *Cercospora* leaf spot of sugar beet showing **A and B**, typical leaf spot in the field, **C**, close-up of individual spots showing the tan-gray centers and brown-purple borders, and **D**, coalescence of spots resulting in tattered appearance of leaves.

the *Cercospora* leaf spot samples are delivered in September. The numbers of samples vary depending on disease pressure, and due to the diligent collection efforts of the grower cooperative agronomists, we tested 1,206 samples in 2002, 934 in 2003, 822 in 2004, 1,096 in 2005, 960 in 2006, 1,036 in 2007, and 899 in 2008 (total = 6,953). Each field sample represents a production field; the samples represent all production areas and all seven factory districts. Additional samples from field fungicide trial plots in the region are also tested for sensitivity to these fungicides, but for this article, only results from the field samples are included; the fungicide trial plot results are excluded from the results. A few samples that are submitted are not tested 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 laboratory, or that the spots were not caused by *C. beticola*.

Triphenyltin hydroxide. Resistance to triphenyltin hydroxide was first reported in 1994, based on growth of *C. beticola* at 1 to 2 $\mu\text{g/ml}$ of this fungicide, and loss of control in the field (6), which continued until 1998 (Fig. 3). Beginning in 1999, the incidence of isolates resistant to triphenyltin hydroxide at 1.0 $\mu\text{g/ml}$ began declining (Fig. 3). In 1998, the percentage of *C. beticola* isolates with resistance to triphenyltin hydroxide, as defined by spore germination at 1.0 $\mu\text{g/ml}$, was 64.6%, decreasing to 0% in 2008 (Fig. 3) (38,45). Based on logistic regression analysis, this is a highly significant reduction of the resistance factor ($P = 0.05$) during the 10-year period from 1998 to 2008. It is interesting to note that this reduction in resistance coincides with registration of additional fungicides and a concomitant reduction in the number of triphenyltin hydroxide applications (Fig. 3). Tetraconazole was first used in 1999, trifloxystrobin in 2002, and pyraclostrobin in 2003. Since the registration of additional fungicides, the number of triphenyltin hydroxide applications has been reduced from an average of 2.14 in 1998 to <1.0 from 2001 through 2008, reducing selection pressure on *C. beticola* to become resistant to triphenyltin hydroxide and reversion from resistance to sensitivity. Such reversion was recently reported in a Japanese population of *Pyricularia*

oryzae to MBI-D fungicides from 78.1% resistance in 2002 to undetectable resistance in 2007 after discontinuance of the fungicide (44). It is also possible that a fitness penalty may be associated with the triphenyltin hydroxide resistance which reduces the ability of triphenyltin hydroxide-resistant *C. beticola* inoculum to survive adverse weather conditions that often occur in the Red River Valley sugar beet production area. In Europe, *C. beticola* isolates with resistance to triphenyltin hydroxide have been reported with reduced competitive ability compared with sensitive isolates (20). Regardless of the mechanism, triphenyltin hydroxide is once again an effective fungicide for managing *Cercospora* leaf spot in North Dakota and Minnesota, and an important partner for managing fungicide resistance. Resistance monitoring, field fungicide trials, and grower fields all attest to the efficacy of triphenyltin hydroxide for *Cercospora* leaf spot control in recent years (Fig. 4).

Thiophanate-methyl. Resistance of *C. beticola* to the benzimidazole fungicide thiophanate-methyl became widespread in the 1980s in many sugar beet production areas of the United States, including the Northern Great Plains (5). In 1998, 70.9% of the samples collected were resistant to thiophanate-methyl as defined by spore germination at 5.0 $\mu\text{g/ml}$; in 1999, 71.3% were resistant; in 2000, 37.0%; in 2001, 56.4%; in 2002, 28.4%; in 2003, 71.3%; in 2004, 78.6%; and in 2007, 41.6%. Resistance to thiophanate-methyl continues to be present in the sugar beet production area of North Dakota and Minnesota, but the percentage of resistant isolates has declined, ostensibly because of less selection pressure as alternate fungicides were registered and used by the sugar beet industry. Thiophanate-methyl is only recommended for application as a tank-mix partner with triphenyltin hydroxide since the percentage of *C. beticola* isolates resistant to both triphenyltin hydroxide and thiophanate-methyl was 2.2% in 2003 and 0.9% in 2004. Thiophanate-methyl applications have declined from 11% of the hectares in 2004 to 4% in 2005 to 1% in 2006.

Triazole. A baseline sensitivity curve was developed for tetraconazole using *C. beticola* isolates from our culture collection (1997 to 1999). Compared to the baseline values, there appears to be an overall doubling of the resistance factor (RF) from 2000 to

Fig. 3. Sensitivity to triphenyltin hydroxide of *Cercospora beticola* isolates collected in North Dakota and Minnesota from 1998 to 2008 at 1.0 $\mu\text{g/ml}$ as measured by bulk spore germination of 100 isolates per field. Blue line shows decline in number of triphenyltin hydroxide applications during this period. Arrows show year of first use of tetraconazole in sugar beet in 1999, trifloxystrobin in 2002, and pyraclostrobin in 2003.

2008. The RF is calculated using the formula $RF = \text{average yearly } EC_{50} \text{ value} / \text{average yearly baseline } EC_{50} \text{ value}$ (48); for the period from 2000 to 2008, $RF = 0.21/0.11 = 1.9$ (Fig. 5). Based on logistic regression analysis of annual EC_{50} values, there is an overall significant increase ($P = 0.05$) in EC_{50} values from 2000 to 2008 compared to the baseline EC_{50} values. In 2002, the first year we found isolates with EC_{50} values $<1.0 \mu\text{g/ml}$, 1.2% of the isolates tested had an EC_{50} value $>1 \mu\text{g/ml}$ to tetraconazole, which increased to 6.0% of the isolates in 2003, 10.8% of the isolates in 2004, 12.4% in 2005, 7.3% in 2006, 9.5% in 2007, and 12.4% in 2008. This also indicates a decrease in sensitivity of *C. beticola* to tetraconazole.

A specific example illustrates the responsiveness that fungicide manipulation can have on *C. beticola* resistance management. The farmers in the Minn-Dak Farmers Cooperative district historically use tetraconazole for the first fungicide application and, due to financial reasons or low disease pressure, may not apply additional fungicides. Consequently, high levels of resistance to tetraconazole ($EC_{50} = 0.25 \mu\text{g/ml}$) compared to other factory districts became a concern to Minn-Dak in 2006 (Fig. 6). The sugar beet industry, including Minn-Dak, is very cognizant of both the economic effect fungicide resistance can have and the necessity of fungicide resistance management because of past *Cercospora* leaf spot epidemics. Consequently, based on discussions between industry and university personnel, the decision was made to not apply tetraconazole in 2007, but instead use alternate fungicides triphenyltin hydroxide and pyraclostrobin. Resistance of *C. beticola* isolates from the Minn-Dak fields in 2007, as measured by EC_{50} values, showed a dramatic reduction in resistance to tetraconazole from $0.250 \mu\text{g/ml}$ in 2006 to $0.081 \mu\text{g/ml}$ in 2007 (Fig. 6). Tetraconazole was used again in 2008 by Minn-Dak, and the EC_{50} value rebounded to $0.228 \mu\text{g/ml}$, clearly showing the effect that fungicide usage has on a fungicide resistance management program.

Sensitivity to two additional triazole fungicides, difenoconazole (Inspire SB) and prothioconazole (Proline), was tested in 2007 and 2008. The average EC_{50} values of *C. beticola* isolates to these two triazole fungicides in 2007 was $0.765 \mu\text{g/ml}$ for prothioconazole

and $0.149 \mu\text{g/ml}$ for difenoconazole compared to $0.212 \mu\text{g/ml}$ for tetraconazole. The percentage of isolates with EC_{50} values of $>1.0 \mu\text{g/ml}$ for these three triazoles was 15.7% for prothioconazole and 9.7% for difenoconazole compared to 12.4% for tetraconazole. While the EC_{50} values of prothioconazole are higher than those of either tetraconazole or difenoconazole, this may be more of a reflection of intrinsic activity of the fungicide rather than higher resistance levels, since there was no loss in disease control in fungicide efficacy trials. The EC_{50} values of prothioconazole decreased to $0.407 \mu\text{g/ml}$ in 2008, while the EC_{50} values for tetraconazole and difenoconazole remained basically unchanged.

QoI. Baseline sensitivity to the QoI (strobilurin) fungicides pyraclostrobin and trifloxystrobin was calculated using *C. beticola* isolates from our culture collection that were not previously exposed to pyraclostrobin and trifloxystrobin (Fig. 7). This baseline was used to monitor shifts in sensitivity to these fungicides in succeeding years. Sensitivity of *C. beticola* to both of these fungicides has remained relatively stable from 2003 to 2008 with only an 8- to 10-fold increase in the resistance factor (RF) compared to the baseline since these fungicides have been used commercially: pyraclostrobin since 2003, trifloxystrobin since 2004 (Fig. 7). It has been demonstrated in *Alternaria solani* that resistance factors less than 10-fold do not affect disease control of QoI fungicides (35,36). Based on logistic regression analysis of annual EC_{50} values for pyraclostrobin, there is an overall significant increase ($P = 0.05$) in EC_{50} values from the baseline of 0.003 compared to the average EC_{50} value in 2008 of 0.033 (Fig. 7). However, substantial variability exists among the isolates tested, with a 1,000-fold difference in EC_{50} values found among the isolates to pyraclostrobin and trifloxystrobin, indicating the potential for reduced sensitivity is present in the population. It should be emphasized that we have found *C. beticola* isolates in the population that have an EC_{50} value $>1.0 \mu\text{g/ml}$ for both pyraclostrobin and trifloxystrobin, a 400-fold increase in the resistance factor. In 2008, only pyraclostrobin was tested at the request of the industry.

It is important to know that there are numerous examples of pathogenic fungi in many crops where resistance has developed to the QoI fungicides due to over application and misapplication of these fungicides, including *A. solani* of potatoes in the midwestern region (36). Because of the widespread application of pyraclostrobin to sugar beets at the end of the season, application on other crops in the sugar beet production area, and the potential for resistance development, it remains critical to monitor sensitivity of *C. beticola* to pyraclostrobin.

Summary, Observations, and Implications of Fungicide Resistance

Triphenyltin hydroxide. Resistance to triphenyltin hydroxide at $1.0 \mu\text{g/ml}$ has almost disappeared in our region, presumably because of the use of alternative fungicides that has resulted in reduction in the number of triphenyltin hydroxide applications from 2.14 in 1998 to less than one each year since 2001 (Fig. 3). In 2008, the number of triphenyltin hydroxide applications was 0.32. In 2006 and 2008, no triphenyltin hydroxide resistant isolates were found. Triphenyltin hydroxide is effective as one of the important fungicides used for *Cercospora* leaf spot control in our area.

Thiophanate-methyl. Resistance to thiophanate-methyl is widespread and is declining slowly. This is probably because thiophanate-methyl usage is also declining. It is only used as a tank mix with triphenyltin hydroxide, and by only a few growers. Thiophanate-methyl resistance appears to be reverting slowly toward sensitivity, but continued application will likely increase the resistance in the population since resistance to thiophanate-methyl tends to be stable for many years and never disappear from a population. A well-documented example is the sustained resistance of *C. beticola* on sugar beet to benzimidazole fungicides in Greece (17).

Triazole. Sensitivity to tetraconazole is relatively stable, but there has been a slow increase in the number of isolates with an $EC_{50} > 1.0 \mu\text{g/ml}$, which indicates the potential for reduced sensi-

Fig. 4. Comparative control of *Cercospora* leaf spot by four applications of triphenyltin hydroxide in A, 1998 and B, 2004, illustrating field control of disease after reversion of resistance.

tivity to develop. In 2006, for the first time since testing began, there was a decrease in both the number of isolates with an EC_{50} value >1.0 $\mu\text{g/ml}$ and the overall EC_{50} value across all isolates tested. In 2008, a decrease in resistance to tetraconazole was observed in five of the seven factory districts. Two additional triazole fungicides, prothioconazole and difenoconazole, were registered for use in 2008 and will compete for the one recommended triazole application per season. Since *C. beticola* has already been shown

to develop cross-resistance to triazole fungicides in Greece (25–27), and historically fungicide resistance of *C. beticola* in the United States follows a similar pattern, special consideration of this fungicide group may be necessary to prevent rapid resistance buildup from overuse and over dependence.

QoI. Sensitivity to pyraclostrobin remains relatively stable, but there are rare isolates identified with a 400-fold increase in resistance factor. There has been a slight increase in the average resis-

Fig. 5. Average effective concentration of tetraconazole at 50% (EC_{50} value) for *Cercospora beticola* isolates collected from 1997 to 2008. There is a significant increase in resistance factor as measured by average EC_{50} values from 2000 to 2008 compared to the baseline EC_{50} values from 1997 to 1999 ($P = 0.05$).

Fig. 6. Sensitivity of *Cercospora beticola* isolates collected from 2005 to 2008 to tetraconazole by factory district. Note the circled Minn-Dak factory district values illustrating the 3 $\times$ changes in resistance factor to tetraconazole from 2006 to 2008.

tance factor (approximately 10×) to pyraclostrobin compared to the baseline since use and testing began in 2003. This change is not a cause for concern due to our previous experiences with this chemistry (35,36), but a few resistant isolates >1 µg/ml were found in the survey, which justifies continued attention. It is critical to continue testing for resistance to pyraclostrobin, since this fungicide is now registered not only for *Cercospora* leaf spot management, but also for plant health purposes, such as increased revenue and increased frost tolerance, even in the absence of *Cercospora* leaf spot (11,12). The widespread and annual choice of pyraclostrobin as the final fungicide applied near the end of the growing season reduces flexibility for fungicide rotation between seasons, especially when only one application may be needed. This, coupled with pyraclostrobin application to other crops grown in our area which may contain weed hosts of *C. beticola*, increases the pressure on *C. beticola* to develop resistance to pyraclostrobin. It should be noted that other research has not shown economic yield benefit by application of fungicides in the absence of disease (22,31).

Success. It appears that the current fungicide resistance management plan we are following is working, since there have been no fungicide failures in our area due to fungicide resistance since 1998 when additional fungicides were registered and used for *Cercospora* leaf spot control. The current program has been successful for 10 years. However, disease pressure has been low the past few years, presumably due to weather conditions unfavorable to *Cercospora* leaf spot development, and higher disease pressure may change fungicide sensitivity patterns. Because *C. beticola* has a history of developing resistance to fungicides and genetic diversity for fungicide resistance (46), the potential for development of resistance to fungicides may always be a factor to consider for disease management. This is especially true since we found both mating types of *C. beticola* naturally occurring in the population in North Dakota and Minnesota (39), but preliminary data do not show a link between mating types and fungicide resistance (3). We must continue to monitor *C. beticola* populations in our area for fungicide sensitivity/resistance and develop disease management strategies with this goal as a high priority to prevent future epidemics and fungicide failures due to fungicide resistance.

Fungicide Resistance Management Recommendations

It is difficult to develop sugar beet hybrids that simultaneously have high resistance to *C. beticola* and high yield potential (42,43). As such, fungicides are generally required to realize high yield potential in areas that provide favorable environmental conditions for development of *Cercospora* leaf spot. *C. beticola* has a history of developing resistance to different fungicides. Therefore, it will continue to be necessary to alternate fungicides (as practiced in the United States) or combine fungicides (as practiced in Europe) with different modes of action to prevent resistance of *C. beticola* to currently registered fungicides. Not only should fungicides be rotated within a season, but between seasons. For instance, if pyraclostrobin were the last fungicide applied the previous year, it should not be the first fungicide applied the current year. In addition to fungicide rotations, there are a number of other research-based recommendations we make to our sugar beet industry in North Dakota and Minnesota for managing fungicide resistance in *C. beticola*. We know that *C. beticola* inoculum that overwinters on debris and soil from the previous crops is the source of infection for the current year (29). We also know that *C. beticola* spores are fragile and do not travel long distances. Therefore, field rotations are an important part of disease control. We recommend a sugar beet crop in a field only every 3 years as the shortest rotation; many growers have a 4-year rotation. Most of our farming is done in quarter-sections (160 acres or 65 hectares), which makes for an easy 4-year rotation for sugar beet.

We recommend using only one triazole fungicide per season and only one QoI fungicide per season, and efficacy trials have consistently demonstrated that a good three-spray program is triazole, triphenyltin hydroxide, and QoI (32). We recommend application of fungicides at full label rates using 187 liters ha⁻¹ of water at a spray pressure of 690 kPa for ground application of fungicides, and 47 liters ha⁻¹ of water for air application. Because all of the fungicides used are protectants, the better the coverage, the better the control. We recommend that the first fungicide application be made at first symptoms, with subsequent applications at least 14 days after the preceding based on the presence of symptoms and daily infection values (DIV), which indicate how favorable the tempera-

Fig. 7. Average effective concentration of trifloxystrobin and pyraclostrobin at 50% (EC₅₀ values) for *Cercospora beticola* isolates collected in Minnesota and North Dakota from 2003 to 2008. Note the circled baseline values of isolates collected prior to use of either fungicide.

Fig. 8. *Cercospora beticola* fungicide sensitivity map illustrating the range of sensitivity to tetraconazole by township in the sugar beet production area of North Dakota and Minnesota. Note clustering (circled) of isolates having a high effective concentration at 50% (EC_{50} values) (0.1 to 1.0 $\mu\text{g/ml}$).

ture and relative humidity are over 48 h for disease development. DIV for two consecutive days ≥ 7 is favorable for disease development (28,40,41). DIVs are provided during the growing season at <http://ndawn.ndsu.nodak.edu/>. Growers at American Crystal Sugar Company and Min-Dak Growers Cooperatives typically follow our recommendations and averaged 1.3 and 0.8 fungicide applications for *Cercospora* leaf spot, respectively, in 2008. Growers at Southern Minnesota, where *Cercospora* leaf spot was most devastating in 1998, typically make three fungicide applications starting at disease onset (9).

It is important to scout at the end of the season to decide the necessity of a late fungicide application; *Cercospora* leaf spot developed later in recent years as a result of lower inoculum pressure and a change in cultivars from the larger bushy triploid cultivars to the more erect diploid cultivars. Cultivars are evaluated for resistance to root diseases and *Cercospora* leaf spot, and growers can only plant cultivars that are approved by the Cooperatives. American Crystal Sugar Company and Minn-Dak Farmers Cooperative approve sugar beet cultivars with a Kleinwanzleber Saat-zucht (KWS) *Cercospora* leaf spot rating scale of 5.2 or less (on a rating scale of 1 to 9, with lower numbers indicating higher *Cercospora* leaf spot resistance). Southern Minnesota Beet Sugar Cooperative, which is the most southern of the three cooperatives and generally has more favorable conditions for *Cercospora* leaf spot, approves cultivars with a KWS rating for *Cercospora* leaf spot of 5.0 and less. Plant resistance helps the fungicide work and reduces fungicide resistance pressure.

A novel contribution to our resistance management program is the use of fungicide resistance maps as an aid for area-wide fungicide selection strategies by the American Crystal Sugar Cooperative for the following year. Each *C. beticola* isolate we receive is uniquely identified by grower and field location, and is individually tested for resistance to multiple fungicides. (In 2008, we tested for resistance to triphenyltin hydroxide, three triazoles, and pyraclostrobin.) The individual isolate $\times$ fungicide sensitivity value is plotted on maps by both township and section. A different map is used for each fungicide, and the sensitivity values are color-coded according to sensitivity category from sensitive (green) to resistant (red); intermediate ratings are yellow and orange. Sensitivity values are estimated based on both field fungicide trials and laboratory testing of *C. beticola* isolates from grower fields and fungicide efficacy trials. The maps are examined for clustering of resistance (Fig. 8). Company agronomists can reference grower fungicide application records and will meet with growers to discuss fungicide application decisions for the next year. If “hot spots” of resistance to a fungicide are identified (Fig. 8), the decision may be made to not apply that particular fungicide the following year or to begin with a fungicide with a different mode of action.

An interesting comment we have heard from the industry is, “is a single spore isolate representative of fungicide sensitivity in an entire field of 135,000 sugar beets per hectare?” In order to at least partially answer that question, we did a small experiment in 2006 to look at the variability in sensitivity to tetraconazole of 10 single-spore *C. beticola* isolates collected from single sugar beet fields in North Dakota and Minnesota. Instead of testing just one single-spore isolate per field for sensitivity to tetraconazole as is our normal practice, for every 100th isolate, we collected 10 single-spore isolates from the bulk spore plate and tested each of the 10 isolates individually for sensitivity to tetraconazole. This was done for nine fields. We calculated an average EC_{50} value for each of the 10 isolates from each field and observed how many of the 10 isolates fell within a resistance factor range $2\times$ above and below the average EC_{50} value. Amazingly, 98% percent of the isolates fell within this $2\times$ range. It appears from this small study that using a single-spore isolate has been representational of an entire field. However, long-term stability of fungicide sensitivity could change because we know that both mating types are present in the population of *C. beticola* in the North Dakota and Minnesota production area.

This story has been a cooperative effort among all of the participants in the sugar beet industry in our growing area and represents

a successful collaboration and team effort to confront and change a fungicide resistance crisis to a fungicide success program. We hope that this case study of success for managing fungicide resistance will serve as an example to other pathogen–fungicide systems and provide inspiration and ideas for long-term disease management by fungicides.

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Dr. Khan is the extension sugarbeet specialist for North Dakota State University and the University of Minnesota. He is responsible for developing, conducting, and evaluating educational programs that will improve sugarbeet production practices in North Dakota and Minnesota. Dr. Khan's re-

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