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Validating the Cercospora Prediction Model as a Decision Making Tool for Managing Cercospora Leaf Spot in Sugarbeet

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INTRODUCTION

The United States is one of the top sugar beet producers in the world (Harveson et al., 2002). Sugar beet was planted on an area of 625,000 ha in 2000, making sugarbeet a major contributor to the U.S. sugar industry. Among the four geographical zones in the U.S., The upper Midwest including Minnesota and North Dakota contributes 48% of the total production of sugar beet (U.S. Department of Agriculture, Economic Research service. 2001).

Cercospora leaf spot caused by *Cercospora beticola* Sacc. is one of the serious diseases of sugarbeet in the United States and in the world (Karaoglanidis et al, 2001, Weiland and Halloin, 2001). In Minnesota and North Dakota, losses in 1998 were estimated at $113 millions from reduced tonnage and increased production cost as a result of fungicides ineffectiveness against *C. beticola* (Cattanach, 2000). Losses of 2-3 tons per acre and of up to 30% in recoverable sucrose under moderate disease conditions has been reported by Khan et al (2001). The disease produces circular spots having ash gray centers with dark brown to reddish purple margins on sugar beet leaves. Favorable environmental conditions for the disease development are day temperatures (25-35^{o}C), night temperature (16^{o}C), and prolonged periods of relative humidity (90-95%) or free moisture on leaves (Shane and Teng, 1984, Windels et al., 1998). Recommended measures for the control of Cercospora leaf spot are application of fungicides, crop rotation and tillage, and planting disease tolerant varieties (Miller et al., 1994).

Currently, sugar beet varieties tolerant to Cercospora leaf spot are grown in the area because resistant varieties have lower sugar yield (Miller et al., 1994). There are four to five genes responsible for *Cercospora* resistance in sugarbeet (Smith and Gaskill, 1970). It is difficult to incorporate *Cercospora* resistance genes into sugarbeet varieties with higher agronomic characteristics because of inbreeding may result in poor plant vigor (Smith and Campbell, 1996). Generally, commercial varieties of sugar beet have tolerance to the disease and require fungicide applications to provide adequate levels of protection against the disease (Miller et al., 1994).

Fungicides based control method during 1999 and 2000 was effective but costly. During 2000, Southern Minnesota Beet Sugar Cooperative growers reported an average of four fungicide applications at an estimated cost of $66.40/acre. Since control of Cercospora leaf spot in sugarbeet is largely dependent upon multiple applications of fungicides, it is imperative to determine the most effective and economical management strategy for controlling the disease without adversely affecting the sugar yield and quality.

OBJECTIVES

1. To evaluate fungicide applications based on the Cercospora leaf spot prediction model.
2. To study the impact of sugarbeet plants age on *C. beticola* infection and disease severity.
3. To explore seasonal availability of *C. beticola* inoculum.
4. To determine the epiphytic nature of *C. beticola* using PCR.

Materials and Methods

Evaluation of fungicide applications based on the Cercospora leaf spot prediction model:

This Research was conducted at Breckenridge, MN, St Thomas, ND and Fargo, ND. Sugarbeet varieties, HH Agate (susceptible to Cercospora leaf spot) and Crystal 222 (tolerant to the disease) were planted at all the locations. The experiment was arranged in a randomized block design with four replications. In Breckenridge, 10 treatments were maintained on each variety which included; 1) untreated control, 2) first application at row closure and continue at 14-day interval until late August or unfavorable conditions for disease development, 3) first application at row closure and repeated once at 21-day interval, third application based on the factory district advisory service indicating high infection possibility, 4) first application at initial symptom and repeated once at 14-day interval, third application based on the factory district advisory service indicating medium infection possibility, 5) first application at initial symptom and repeated once at 14-day interval, third application based on DIVs ≥7 for 2 consecutive days at 87% RH (using Hobo's), 6) first application at initial symptom and repeated once at 14-day interval, third application based on DIVs ≥7 for 2 consecutive days at 90% RH (using Hobo's), 7) first application at initial symptom and subsequent applications according to DIVs ≥7 for 2 consecutive days at 90% RH (using Hobo's), 8) first application at initial symptoms and subsequent applications according to DIVs ≥7 for 2 consecutive days at 87% RH (using Hobo's), 9) first application according to DIVs ≥7 for 2 consecutive days at 87% RH (using Hobo's) and subsequent applications after 14 days according to DIVs ≥7 for 2 consecutive days at 87% RH (using Hobo's), and 10) first application according to DIVs ≥7 for 2 consecutive days at 90% RH (using Hobo's) and subsequent applications after 14 days according to DIVs ≥7 for 2 consecutive days at 90% RH (using Hobo's). In St. Thomas, five treatments were maintained on each variety which included; 1) untreated control, 2) first application at initial symptom and repeated once at 14-day interval, third application based on the factory district advisory service indicating high infection possibility, 3) first application at initial symptoms and subsequent applications according to DIVs ≥7 for 2 consecutive days at 87% RH (using Hobo's), 4) first application at initial symptoms and subsequent applications according to DIVs ≥7 for 2 consecutive days at 90% RH (using Hobo's), and 5) first and subsequent applications at 14-day interval based on DIVs ≥7 for 2 consecutive days at 87% RH (using Hobo's).

Seasonal availability of *C. beticola* inoculum: Three experiments were conducted for the exploration of primary inoculum availability and its subsequent dissemination in the sugarbeet field.
i. Viability of Cercospora spore in the field: In this experiment sugarbeet leaves having Cercospora leaf spots were put in ladies pantyhose and then sealed it in sacks made of window screen (fiber glass). Objective of this experiment is to study for how long the Cercospora spores would be viable in the natural condition in the field. These sacks were then buried in the ground for 1, 2 and 3 years. The treatments were replicated three times and included; 1) put the sacks on the ground, 2) buried 4 inches in the ground, and 3) buried 8 inches in the ground. Each year these sacks will be checked for the viability of the inoculum.
ii. Sources of primary inoculum for disease initiation: This experiment was conducted at Breckenridge experimental field. Objective of this experiment was to study possible sources of primary inoculum in the field for the disease initiation. Randomized complete block design was followed, and each treatment was replicated three times. Treatments included; 1) covered sugarbeet plants with plastic cages, 2) covered sugarbeet plants with plastic cages and covered the ground with plastic between the plant's rows, 3) covered the ground with plastic between the plants rows, and 4) untreated check. This experiment was started in July, 2002 and continued till harvesting. The data on disease severity following KWS rating scale (1-9) was recorded in September 2002.
iii. Movement of Cercospora spores in the field: This experiment was conducted at Breckenridge, Fargo and St. Thomas with the objective to find out time of Cercospora spores production, development and dissemination in the field, and their possible relationship with the disease development. Spore traps made of coffee cans were placed in the field one and two feet above the ground. Microscopic slides covered with petroleom jelly on one square inch area were fixed in those cans with the help of clamps. Each can contained two slides facing opposite directions. Each field had 8 slides except St. Thomas (4 slides) facing four directions. The slides were replaced weekly and examined for number of spores trapped per week under the microscope. This experiment was started in the last week of June, 2002 and continued until the middle of September 2002.

Epiphytic nature of *C. beticola*: Objective of this experiment was to find out the epiphytic nature of the pathogen. Four sugarbeet fields were selected at Breckenridge in the first week of July 2002. Two of them were planted late (plants were smaller), and two were planted at normal time (plants were near to row closure). Twenty samples, each sample contained two fully expanded leaves, were collected weekly from each field and put in paper bags separately. These samples were then frozen in $-80^{o}C$ freezer until to examine for the presence of Cercospora spores using molecular technique. Collection of samples was continued for six week till to first week of August 2002. Cercospora DNA was isolated from each sample, run it on 1% agarose gel, and compared the band with the positive band of Cercospora.

Summary of Results

Spore traps

The number of spores trapped was highest in late August and early September that corresponds to the time that Cercospora leaf spot symptom was most prevalent. The Burkard trap was more effective than the hand-made trap. More spores were trapped at the Cercospora disease nursery at Crookston where disease severity was significantly higher than Breckenridge. Spore count can be a useful tool in managing Cercospora leaf spot with fungicides.

Cercospora Prediction Model and Fungicide Applications

Results for the area (St. Thomas) north of the Norman-Polk county line was consistent with the recommendation of applying the first fungicide application at first symptoms and subsequent applications based on DIV and disease severity. Two fungicide applications were necessary for effective Cercospora leaf spot control on a susceptible and a tolerant variety tested at St. Thomas.

For the area (Breckenridge) south of the Norman-Polk county line, our results show that one fungicide application at first symptoms was as effective as two applications, since the plots were replanted late (June 5) and disease severity was low in the plots late in the season after the first fungicide application. Recoverable sucrose per acre in the untreated check was not significantly lower than the treated plots because infection occurred late in the season and disease severity was low.

Table 1. Cercospora leaf spot control using the Prediction Model at Breckenridge, 2002

Treatment	Recoverable Sucrose		Root Yield	Sucrose Content	CLS*	Ret-Urns
	(lb/A)	(lb/T)	(T/A)	(%)		$
HH Agate - Susceptible						
Untreated Check	4308e	239ef	18.4de	14.2d	2.5bc	
Row Closure/ 14 d interval	4629b-e	250b-f	18.9b-e	14.7bcd	2.3abc	
Row closure/ 21 d after/FDAS (high)	4352e	240ef	18.5cde	14.3cd	2.0ab	
0.01% disease/ 14 d after/FDAS (med)	4720b-e	249c-f	19.2a-e	14.7bcd	2.0ab	
0.01% disease/ 14d/DIV≥7, RH 87%	4556de	242ef	19.2a-e	14.3cd	2.0ab	
0.01% disease/ 14d/DIV≥7, RH 90%	4661b-e	244ef	19.5a-e	14.3cd	2.0ab	
0.01% disease/ DIV≥7, RH 90%	4514de	247ef	19.3a-e	14.2d	2.8c	
0.01% disease/ DIV≥7, RH 87%	4579cde	236f	19.8a-e	14.2d	2.7c	
DIV≥7, RH 87%/ 14d (Hobo)	4718b-e	248def	19.4a-e	14.6bcd	2.7c	
DIV≥7, RH 90%/ 14d (Hobo)	4552de	245ef	18.9b-e	14.4cd	2.8c	
Crystal 222- Tolerant						
Untreated Check	5067a-d	282a	18.2e	15.9a	2.5bc	
Row Closure/ 14 d interval	5606a	273ab	20.8a	15.5ab	1.8a	
Row closure/ 21 d after/FDAS (high)	5247abc	274a	19.4a-e	15.6ab	2.0ab	
0.01% disease/ 14 d after/FDAS (med)	5159a-d	274a	19.0a-e	15.5ab	2.3abc	
0.01% disease/ 14d/DIV≥7, RH 87%	5512a	276a	20.3abc	15.6ab	2.3abc	
0.01% disease/ 14d/DIV≥7, RH 90%	5516a	276a	20.2a-d	15.6ab	2.3abc	
0.01% disease/ DIV≥7, RH 90%	5309ab	272a-d	19.8a-e	15.2abc	2.5bc	
0.01% disease/ DIV≥7, RH 87%	4945a-e	261a-e	19.2a-e	15.0a-d	2.0ab	
DIV≥7, RH 87%/ 14d (Hobo)	5062a-d	261a-e	19.7a-e	14.9a-d	2.5bc	
DIV≥7, RH 90%/ 14d (Hobo)	5507a	273abc	20.5ab	15.4ab	2.3abc	
LSD (P=0.05)	686	24	1.8	1.0	0.6	
CV%	9.9	6.6	6.7	4.9	18.1	

*Cercospora leaf spot measured on KWS scale 1-9 (no leaf spot – dead outer leaves, inner leaves severely damaged, regrowth of new leaves)

Table 2: Cercospora leaf spot control using the Prediction Model at St. Thomas, 2002

Treatment	Recoverable Sucrose		Root Yield	Sucrose Content	CLS*	Ret-urn
	(lb/A)	(lb/T)	(T/A)	(%)		$
HH Agate – Susceptible						
Untreated Check	4719bc	266a	18.1bc	15.1a	7.3d	
0.01% disease/ 14 d after	5372a	258a	21.3a	14.6a	2.5ab	
0.01% disease/ 14d/ DIV≥7, RH 90%	5351a	256a	21.4a	14.6a	3.0b	
0.01% disease/ 14d/ DIV≥7, RH 87%	5145ab	257a	20.3a	14.8a	2.8ab	
DIV≥7, RH> 87%	4639bc	261a	18.1bc	14.8a	5.5c	
Crystal 222 – Tolerant						
Untreated Check	4537c	261a	17.6c	14.7a	5.0c	
0.01% disease/ 14 d after	5034abc	253a	20.2a	14.5a	2.0a	
0.01% disease/ 14d/ DIV≥7, RH 90%	5518a	272a	20.4a	15.2a	2.0a	
0.01% disease/ 14d/ DIV≥7, RH 87%	5156ab	263a	19.8ab	14.9a	2.0a	
DIV≥7, RH> 87%	4774bc	263a	18.4bc	14.9a	5.3c	
LSD (P=0.05)	536	31	1.8	1.3	0.96	
CV%	7.31	8.2	6.2	6.0	17.9	

*Cercospora leaf spot measured on KWS scale 1-9 (no leaf spot – dead outer leaves, inner leaves severely damaged, regrowth of new leaves)

Table 3: Cage study

Treatments	Average disease severity*
No ground cover and cage (Check)	3.5b
Cover ground with plastic	3.7b
Plastic cage around the plants and cover ground with plastic	2.2a
Plastic cage around the plants	2.1a
LSD (P=0.05)	1.1
CV%	18.4

**Cercospora leaf spot measured on KWS scale 1-9 (no leaf spot – dead outer leaves, inner leaves severely damaged, regrowth of new leaves)

Table 4: Cercospora spores trapped in the sugarbeet field at Fargo using self- made spore traps

Date	Spores trapped	Average weekly environmental data		
		Temperature	% RH	Leaf Wetness
6/27/02	0	72.9	76.0	2.6
7/4/2	0	77.3	58.8	5.8
7/13/2	0	72.3	78.8	1.7
7/21/2	0	77.3	78.7	2.9
7/27/2	0	68.9	80.7	6.9
8/2/2	0	69.9	71.4	3.4
8/9/2	0	70.3	74.5	1.0
8/16/2	5	66.0	73.4	1.1
8/22/2	3	63.5	78.0	2.1

8/29/2	0	73.0	76.4	2.0
9/4/2	8	70.7	75.0	0.9
9/12/2	0	72.4	68.0	1.3
9/17/2	1	64.4	58.7	0.0

Table 5: Cercospora spores trapped in the sugarbeet field at Breckenridge using self-made spore traps

Date	Spores trapped	Average weekly environmental data		
		Temperature	% RH	Leaf Wetness
6/27/02	0	73.7	75.9	0.9
7/4/2	0	78.2	57.7	0.1
7/13/2	3	72.6	80.4	2.1
7/21/2	0	76.2	83.4	1.9
7/27/2	4	68.5	84.6	2.9
8/2/2	0	69.2	79.4	2.3
8/9/2	9	69.1	84.1	2.2
8/16/2	0	64.9	83.7	2.4
8/22/2	2	62.4	86.0	2.6
8/29/2	17	71.8	85.8	4.9
9/4/2	1	69.1	83.9	2.0
9/12/2	20	70.6	78.6	2.0
9/17/2		69.7	67.7	1.0

Table 6: Cercospora spores trapped in the sugarbeet field at St. Thomas using self-made spore traps

Date	Spores trapped	Average weekly environmental data		
		Temperature	% RH	Leaf Wetness
7/4/2	0	75.1	62.2	0.6
7/13/2	0	71.3	74.7	1.0
7/21/2	0	75.8	79.7	1.5
7/27/2	0	69.3	74.1	0.6
8/2/2	0	68.5	68.3	1.3
8/9/2	1	68.1	80.9	2.2
8/16/2	0	64.3	84.3	3.7
8/22/2	0	61.9	79.8	1.5
8/29/2	4	69.8	81.8	2.9
9/4/2	50	69.1	79.5	2.4
9/12/2	35	66.1	80.4	1.6
9/17/2	47	64.1	63.2	0.0

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