# Fluctuations in Number of *Cercospora beticola* Conidia in Relationship to Environment and Disease Severity in Sugar Beet

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### ABSTRACT

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Cercospora leaf spot, caused by *Cercospora beticola*, is the most damaging foliar disease of sugar beet in Minnesota (MN) and North Dakota (ND). Research was conducted to characterize the temporal progression of aerial concentration of *C. beticola* conidia in association with the environment and disease severity in sugar beet. In 2003 and 2004, volumetric spore traps were placed within inoculated sugar beet plots to determine daily dispersal of conidia at Breckenridge, MN, and St. Thomas, ND. Plots were rated weekly for disease severity. At both locations, conidia were first collected in early July 2003 and late June in

Minnesota (MN) and North Dakota (ND) produced 57% of the total U.S. sugar beet (*Beta vulgaris* L.) production in 2007 (23). The sugar beet industry contributes \$3 billion in total economic activity in MN and ND (1), and is particularly important in the economy of rural communities. One of the limiting factors for sugar beet production in the United States and worldwide (10) is the damaging foliar disease, Cercospora leaf spot.

Cercospora leaf spot is managed using an integrated system that includes cultural practices such as rotation with nonhost crops (44) and tillage, planting moderately resistant cultivars (22, 30,31,36,37), and fungicide applications (13,16,43,44). It is difficult to breed the four to five genes responsible for Cercospora resistance (37) into cultivars that will produce high sugar yields (36). As such, in North Dakota and Minnesota, commercial cultivars typically require two to four fungicide applications annually (4,6). Growers apply fungicides based on a number of factors including row closure when leaves from adjoining rows are touching, appearance of symptoms, weather conditions, and daily infection values available on the web at http://ndawn.ndsu.nodak.edu based on the Cercospora management model (13,43). Cercospora beticola Sacc., the causal agent of Cercospora leaf spot in sugar beet, was first reported and characterized by Saccardo (33), and is considered to originate in central Europe and the Mediterranean area (9). The fungus reproduces through asexual conidia and no confirmed sexual stage has been reported.

Conidia of *C. beticola* penetrate through stomata into the parenchymatous leaf tissues and grow intercellularly (28,38). Symptoms develop within 5 to 21 days after infection depending on

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2004. Peaks of conidia per cubic meter of air were observed with maxima in late August 2003 and in early September 2004 at both locations. Peaks of airborne conidium concentration were significantly correlated with the average temperature of daily hours when relative humidity was greater than 87%. Weekly mean hourly conidia per cubic meter of air was significantly (P < 0.01) associated with disease severity during both years and across locations. This study showed that *C. beticola* conidial numbers may be used to estimate potential disease severity that, with further research, could be incorporated in a disease forecasting model to rationalize Cercospora leaf spot management.

Additional keywords: Beta, polycyclic, sporulation.

weather conditions (43). *C. beticola* produces circular necrotic spots about 3 to 5 mm in diameter with tan to ash gray centers surrounded by dark brown to reddish purple margin on sugar beet leaves (11,12,18,27). Conidia are produced on the surface of necrotic spots projecting as darkly pigmented structures called pseudostromata that are scattered throughout the center of the lesion (38). Sporulation of the fungus occurs extensively 3 days after necrosis of the infected tissues (31).

The fungus is polycyclic within a beet growing season. One cycle of sporulation typically takes 12 days depending on field conditions (31,41). Conidia of *C. beticola* are produced most readily at temperatures from 15 to  $23^{\circ}$ C and relative humidity (RH) greater than 60%, but do not form at temperatures less than 10°C or above  $38^{\circ}$ C (26). Favorable environmental conditions for Cercospora leaf spot development are day temperatures of 25 to  $35^{\circ}$ C, night temperature of  $16^{\circ}$ C, and prolonged periods of RH of 90 to 95% or free moisture on leaves (7,32,34).

Conidia of C. beticola are dispersed from their source of inoculum by wind, water splash, running water, and insects (5, 20,21). However, wind has been considered the major component of C. beticola dispersal (15,19,21). Airborne conidia of C. beticola play an important role in primary infection, secondary infection, progressive increase in disease development, and ultimately the spread of disease epidemics in a region. Given the key role played by airborne conidia in disease spread, knowledge of temporal dispersal of C. beticola inoculum during the sugar beet growing season will provide useful information that could be used to improve disease management. The objectives of this study were (i) to characterize temporal progression of aerial concentration of C. beticola conidia; (ii) to determine the relationship between aerial concentration of C. beticola conidia and environment; and (iii) to evaluate the relationship between aerial concentration of C. beticola conidia and disease severity in sugar beet at research sites in Minnesota and North Dakota.

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### MATERIALS AND METHODS

Daily pattern of C. beticola conidia at research sites. In 2003 and 2004, research was conducted in field plots  $(13.5 \times 9 \text{ m})$ planted with sugar beet cultivar Beta 3800, susceptible to Cercospora leaf spot (24), at Breckenridge, MN, and St. Thomas, ND. Crops were sown at both locations in the first week of May 2003 and in the last week of April 2004. Seeds were drilled using a commercial planter into rows 0.55 m wide and 9.0 m long. Terbufos (Counter 15G, BASF, Raleigh, NC) was applied modified in-furrow at planting at a rate of 3.7 kg a.i. ha<sup>-1</sup> to control sugar beet root maggot (Tetanops myopaeformis Von Röder) (2). Weeds were controlled using recommended herbicides (14) and hand weeding. At the four-leaf stage, populations were thinned to 86,450 plants ha<sup>-1</sup>. Both locations were inoculated with C. beticola inoculum in the first week of June during both years. Rows one and six of six-row plots were manually inoculated using dried infected leaves obtained from Betaseed nursery in Shakopee, MN, where no fungicides were applied. Leaves were mixed with talc (2:1 by weight) and applied at  $5.6 \text{ kg ha}^{-1}$ .

At both locations, volumetric spore traps (Osborne Applied Science, White, SD) were set up in the center of a sugar beet plot after inoculation and monitored until 13 September. The volumetric spore traps were programmed to aspirate at a rate of 10 liters per min. The traps were set to collect conidia for up to 7 days at a time, usually starting and ending at 1200 h. Sticky tape exposed in sugar beet field plots for 1 week was cut into seven equal sections; each section represented 1 day. Each section was examined microscopically at 250× after staining with lactophenol aniline blue for counting the daily number of C. beticola conidia. Daily spore count was converted to concentration of spores per cubic meter of air per hour by dividing the total daily spore count (that is, the daily mean hourly concentration – spores  $m^{-3} h^{-1}$ ) by the total air volume throughput ((0.6 m<sup>3</sup> h<sup>-1</sup>)  $\times$  24 h). Weekly total spore count was converted to weekly mean hourly concentration of spores per cubic meter of air per hour by dividing the total weekly spore count with total air throughput  $((0.6 \text{ m}^3 \text{ h}^{-1}) \times 168 \text{ h})$ .

Weekly pattern of Cercospora leaf spot severity at research sites. The research plots with spore traps were rated weekly for Cercospora leaf spot disease severity in 2003 and 2004 at Breckenridge, MN and St. Thomas, ND. The main plot  $(13.5 \times 9)$ m) was divided into four subplots  $(3.3 \times 9 \text{ m})$ , each with six rows of sugar beet, to represent four replicates of the disease severity ratings. Ratings were done from early June to 13 September. The middle two rows of each plot were rated weekly for Cercospora leaf spot severity using the Kleinwanzlebener Saatzucht (KWS) scale (15), ranging from 1 to 9, in which 1 = no Cercospora leaf spot symptoms, 3 = leaf spots on the older leaves, 5 = leaf spotscoalescence to form small necrotic areas, 7 = death of older leaves and leaf spot progression to the inner leaves, and 9 = death of all leaves and initiation of new foliage. The rating scale numbers were transformed to the spot percentage scale (1 to 10) developed by Shane and Teng (35). The spot percentage disease severity allowed for measuring disease severity at low and high intensities and has been used in the prediction model to forecast Cercospora leaf spot in the sugar beet growing regions of MN and ND (34).

**Meteorological measurements.** Environmental variables were reported from 21 June through 13 September in 2003 and 2004 at Breckenridge, MN and St. Thomas, ND. Data loggers (HOBO, Onset Computer Corporation, Bourne, MA) were set up at each location to record air temperature (°C) and RH (%) under the sugar beet canopy. The data loggers were set to take readings at 1-h intervals.

**Data analysis.** RH of 87% or higher was important since it was considered as one of the necessary factors, along with favorable temperature, for infection by the pathogen (11). The exponential equation has been used to describe both the relationship between

the spore concentrations of C. beticola and the mean temperature during the hours when the RH was greater than 87% and the relationship between the disease severity and the accumulated weekly hourly spores using the standard nonlinear curves of GenStat Release 10 (VSN International, Hemel Hempstead, UK). For the former relationship, a double exponential equation might be more appropriate for the response to the full temperature range from 10 to 38°C of the production in conidial numbers in order to describe an exponential increase of spore concentration to an optimum temperature and then an exponential decline of spore concentration to a maximum critical temperature. However, the apparent optimal temperature for spore productions at about 25°C was not transgressed in the data set collected in this study, so the simple exponential function to the left was fitted to the data. For describing the relationship of percentage Cercospora disease severity with the accumulated weekly mean hourly spores, the logistic model should be more appropriate because it is able to incorporate the upper asymptote which represents the maximum level of the disease severity when increases of Cercospora spores beyond this point have had little effects on the increase of disease severity. Examination of the observations has not seen the asymptotic leveling off, thus, the simple exponential equation was again fitted to these data. Parallel curve analysis was conducted to examine the effects of years and sites.

The variance accounted for  $(R^2)$  and the significant level for the *F* test were calculated to indicate the goodness of fit of the exponential equation. The variance accounted for is a measure of how well the equation describes the data. The significant level is the probability of being wrong in concluding that there is an association between the dependent and the independent variables. If the calculated significant level is greater than 5%, there is then no association between the dependent and the independent variables.

# RESULTS

In 2003, conidia of *C. beticola* were first trapped on 5 and 3 July at Breckenridge, MN and St. Thomas, ND, respectively (Fig. 1). Daily number of spores per cubic meter of air increased with time until the highest numbers were reached on 24 and 25 August at Breckenridge, MN and St. Thomas, ND, respectively, and then decreased through 13 September. In 2004, conidia were first trapped on 22 and 24 June at Breckenridge, MN and St. Thomas, ND (Fig. 1), respectively. Daily number of spores per cubic meter of air increased gradually with time, and the highest numbers were attained on 2 September at Breckenridge, MN and on 4 September at St. Thomas, ND (Fig. 1). Several peaks of conidial production were observed at all locations during both years.

Significant exponential relationships between peaks of the daily mean hourly number of spores per cubic meter of air and average air temperature of daily hours when RH was greater than 87% were observed across locations and years except at St. Thomas in 2004 (Fig. 2). As the average temperature of daily hours when RH was higher than 87% increases, the daily hourly mean conidia trapped increased. However, there was significant difference (P <0.05) between years at Breckenridge, MN in the increases of the spores trapped with the increasing average temperatures. The difference was also significant (P < 0.05) between Breckenridge, MN and St. Thomas, ND in both 2003 and 2004 in the increases of the spores trapped with the increasing average temperatures (Fig. 2). Although, the fitted exponential relationship was not statistically significant (P > 0.15) with the observations alone at St. Thomas in 2004, the fitted exponential equation with observations in 2003 was adequate to describe those observations in 2004 (Fig. 2). In 2003, Cercospora leaf spot was first observed on 19 and 26 July at Breckenridge, MN and St. Thomas, ND, respectively. In 2004, Cercospora leaf spot was first observed on 26 July

at both locations. When the accumulated weekly mean hourly concentration of airborne conidia per cubic meter reached one, first symptoms of the disease were observed in surrounding plots 14 and 21 days later in 2003 and 2004, respectively. The percentage disease severity increased with an increase in the accumulated weekly mean hourly concentration of airborne conidia per cubic meter. Disease severity was significantly associated with the accumulated weekly mean hourly concentration of airborne conidia per cubic meter at all locations during both years (Fig. 3). In 2003, a significantly (P < 0.001) exponential association was identified between disease severity and the accumulated weekly mean hourly concentration of airborne conidia per cubic meter at Breckenridge, MN and St. Thomas, ND with the variance accounted for  $(R^2)$  that were 99.7 and 99.3%, respectively (Fig. 3). In 2004, there was again a significant (P < 0.001) exponential association between disease severity and the accumulated weekly mean hourly concentration of airborne conidia per cubic meter at both Breckenridge, MN and St. Thomas, ND with the variance accounted for  $(R^2)$  that were 92.4 and 97.2%, respectively (Fig. 3). Parallel curve analysis has indicated significant differences (P < 0.05) between years in the fitted relations between the percentage disease severity and the accumulated weekly mean hourly concentration of airborne conidia per cubic meter at both locations and also between locations in both years.

# DISCUSSION

This is the first report of the seasonal pattern of *C. beticola* conidial dispersal and its association with Cercospora leaf spot disease severity in sugar beet in Minnesota and North Dakota.

The presence of inoculum and favorable environmental conditions are major components for disease epidemics. The study on the daily assessment of C. beticola conidia in sugar beet plots showed the dynamics of inoculum production during the sugar beet growing season in relation to environmental variables. All plots at both sites had been inoculated with the same mass of leaf material infected with C. beticola inoculum. However, the amount of viable inoculum was not measured, and thus it was assumed that the primary inoculum biomass was evenly distributed over leaf mass. Initially, the number of conidia trapped was low but with time increased exponentially to a maximum level, and then decreased at the end of the season. However, there were a lot of fluctuations in spore production during the season. On some days a few or no conidia were trapped, on other days more conidia were trapped. Low number of conidia may be attributed to exhaustion of the sources of inoculum and/or unfavorable environmental conditions and high number of conidia may be a result of favorable conditions for sporulation. Several peaks of conidial production were observed from July through 13 September, indicating multiple cycles of sporulation during the sugar beet growing season (Fig. 1). The polycyclic nature of the pathogen results in new infections (i.e., secondary infection). While primary infections are mainly related to the initial viable amounts of inoculum sources, secondary infections are more determined by such environmental conditions as wind, rainfall, humidity, and temperature, all of which interact to affect the production of spores and the spread of them. In the greenhouse, symptoms developed from 7 to 10 days postinoculation (40). However, it takes 3 to 4 days for the new spots to produce new conidia (31,42), which may become new sources of inoculum.

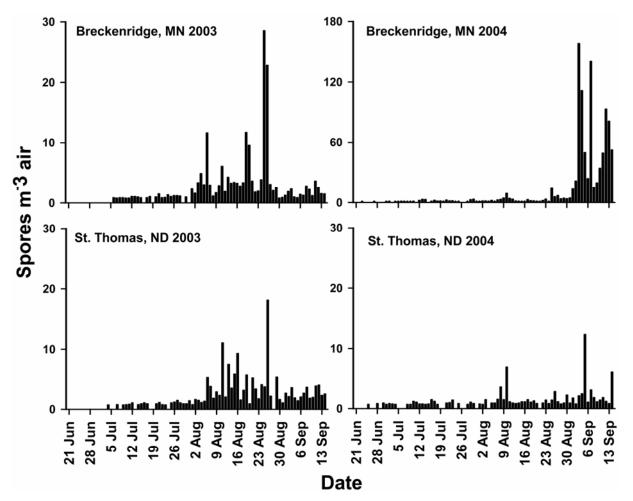
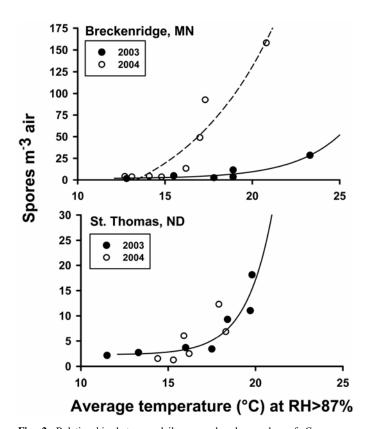


Fig. 1. Daily number of *Cercospora beticola* spores  $m^{-3}$  of air collected with volumetric spore traps in sugar beet fields during 2003 and 2004 at Breckenridge, Minnesota (MN) and at St. Thomas, North Dakota (ND).

In this study, interaction of temperature and RH contributed to high production of C. beticola conidia. Peaks of conidial dispersal pattern were positively associated with the average temperature of daily hours when RH was greater than 87%. It was apparent that below 10°C, no conidia were trapped. However, as the temperature increased and RH was greater than 87%, there was a steady increase in the production of C. beticola conidia. Our results are similar with those of Pool and McKay (26) except they reported RH greater than 60% was favorable for higher conidial production rather than RH greater than 87%. This difference might be because of the different methods used to study conidial production. They studied the production of conidia on old and new leaf surface in relation to environmental conditions, whereas we determined sporulation patterns using spore traps. During spore trapping, other environmental variables such as wind velocity and rainfall were involved that could directly or indirectly impact collection of spores in the field. In a similar study on C. zeaemaydis causing gray leaf spot of maize, Paul and Munkvold (25) reported association of high sporulation of the fungus with the interaction of temperature and 100% RH.

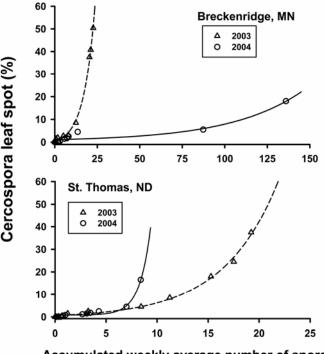
The percentage of Cercospora disease severity was transformed from the KWS scale scores (17) following the method by Shane and Teng (34,35). This involved the change in nature of the disease severity from measuring plant organ death to measuring disease density. The KWS scale score measures are not only affected by the direct impact of the environment on plant physiology but also by the interaction between plant physiology,



**Fig. 2.** Relationship between daily mean hourly number of *Cercospora beticola* spores m<sup>-3</sup> of air and average temperature (°C) of daily hours when relative humidity (RH) was greater than 87% in sugar beet fields in 2003 and 2004 at Breckenridge, Minnesota (MN) and at St. Thomas, North Dakota (ND). Each data point represents peak of daily hourly concentration of *C*. *beticola* spores per cubic meter of air. The fitted exponential equations at Breckenridge, MN are  $y = 1.59 + 0.0049 \times 1.447^x$  with  $R^2 = 86.5\%$  and P < 0.05 in 2003, and  $y = -53.5 + 4.50 \times 1.204^x$  with  $R^2 = 87.0\%$  and P < 0.01 in 2004. The fitted exponential equation at St Thomas, ND is  $y = 2.28 + 0.000025 \times 1.944^x$  with  $R^2 = 81.3\%$  and P < 0.05 in 2003. In 2004, the relationship between daily mean hourly number of *C. beticola* spores/m<sup>3</sup> of air and average temperature was not significant (P > 0.15).

disease and the environments. This should act to weaken the direct relationship between airborne spore concentration and the disease severity. Nonetheless, this research showed a significant relationship between *C. beticola* conidial concentration and percentage Cercospora leaf spot severity. Our results were similar to Rodriguez et al. (29), who showed a strong relationship of *Helminthosporium solani* Durieu and Mont. spores in the storage period and infection of potato (*Solanum tuberosum*) tubers in stores. Association of airborne conidial concentration and disease severity was also reported in studies on *Botrytis squamosa* Walker in onions, *Allium cepa* L. (3).

The exponential equation that described the relationship between percentage disease severity and inoculum showed a sharp increase in percentage disease severity when the accumulated weekly mean hourly conidial concentration in the air were higher than 5 spores m<sup>-3</sup>; whereas, when accumulated weekly mean hourly conidial concentration was under 4 spores/m<sup>3</sup>, disease severity was lower than 1%. In this research, the fitted exponential equations explained more than 98% variation observed in the field when the disease pressure was higher in 2003 and more than 90% variation observed in the field when the disease pressure was lower in 2004 (Fig. 3). However, the fitted equations differed significantly in their parameter estimates both between years at a given site and between sites in a given year. This could result from the differences in environmental conditions which affected the disease development in combination with airborne conidial concentration of C. beticola. For example, in 2004 at Breckenridge, MN and St. Thomas, ND, average daily temperature was unusually low and much below the normal average temperature (data not shown) from 9 August to 23 August which adversely affected the Cercospora leaf spot development in sugar



Accumulated weekly average number of spores

**Fig. 3.** Relationship between accumulated weekly mean hourly number of *Cercospora beticola* spores/m<sup>3</sup> of air starting from 5 July and percentage of Cercospora leaf spot severity in sugar beet in 2003and 2004 at Breckenridge, Minnesota (MN) and at St. Thomas, North Dakota (ND). Each data point is the mean of four observations. The fitted exponential equations at Breckenridge, MN are  $y = -1.19 + 1.585 \times 1.1671^x$  with  $R^2 = 99.7\%$  and P < 0.001 in 2003, and  $y = 0.381 + 0.714 \times 1.0283^x$  with  $R^2 = 92.4\%$  and P < 0.001 in 2004. The fitted exponential equations at St Thomas, ND are  $y = -0.473 + 1.045 \times 1.2039^x$  with  $R^2 = 99.3\%$  and P < 0.001 in 2003, and  $y = 0.796 + 0.00556 \times 2.574^x$  with  $R^2 = 97.2\%$  and P < 0.001 in 2004.

beet fields. It is possible that in some instances environmental conditions may be favorable for sporulation but may not be conducive for infection and disease development. For example, conditions of 15 to 23°C and RH greater than 60% are favorable for sporulation (18) but if the temperature just after sporulation dropped to below 14°C, *C. beticola* infection and disease development would be highly unlikely.

Seasonal pattern of inoculum dispersal in the field using spore traps measures the actual presence of inoculum, and can be used to estimate potential disease severity. Forecasting systems have been developed to estimate inoculum production based on the weather variables for many plant pathogens including apple scab (8) and onion leaf blight (39). These models estimate the potential for inoculum development, not the actual presence of inoculum. However, this study showed the actual presence of inoculum development, and its relationship to environmental conditions and disease development. It may be possible to incorporate the airborne concentration of *C. beticola* conidia into the Cercospora prediction model developed by Shane and Teng (34) to assist growers particularly in making the first fungicide application because spore concentration is an important component of the input variables.

Increase of *C. beticola* conidia temporally in sugar beet fields and its association with disease development in the presence of favorable environmental conditions provide an opportunity to further improve the Cercospora management model (13) by possibly using the presence and number of conidia to determine when to scout fields for disease symptoms. We now have the ability to estimate the levels of Cercospora disease severity using the spore concentration, but we still are unable to know when the disease symptoms will actually appear. So, field scouting is still necessary to determine the most appropriate time for fungicide applications. Further field research could be done to improve timing of fungicide applications based not only on appearance of symptoms and environmental conditions, as is currently recommended, (13) but also on spore concentration and/or days after specific spore concentrations are recorded.

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