PREVALENCE, DIVERSITY, AND MANAGEMENT OF GOSS'S LEAF BLIGHT

(*CLAVIBACTER NEBRASKENSIS*) IN NORTH DAKOTA

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Elizabeth Crane Bauske

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Prevalence, Diversity, and Management of Goss's Leaf Blight
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The Supervisory Committee certifies that this ***disquisition*** complies with North Dakota

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DOCTOR OF PHILOSOPHY

SUPERVISORY COMMITTEE:

Dr. Andrew Friskop

Chair

Dr. Julie Pasche

Dr. Zhaohui Liu

Dr. Joel Ransom

Approved:

November 8, 2018	Dr. Jack Rasmussen
Date	Department Chair

ABSTRACT

Goss's wilt and leaf blight (GWLB) (*Clavibacter nebraskensis*) is a yield-limiting disease of corn (*Zea mays* L.). Research conducted in other corn growing states have indicated yield losses as severe as 60% on susceptible hybrids. In 2011, the disease was first reported in southcentral North Dakota (ND). Concurrently, corn production was increasing in ND due to favorable grain prices. With increased production, there was concern about disease prevalence and the impact of GWLB on corn yields in the state. In order to determine which corn diseases are present in ND, and to gain a better understanding of the *C. nebraskensis* population in ND and its impact on corn yield, three studies were conducted. Prior to 2014, no formal corn foliar disease survey had been documented in ND, thus the first objective was to document the prevalence of foliar diseases of corn in ND. Results indicate that four diseases are common in ND; common rust, common smut, northern corn leaf blight, and GWLB. One corn disease is of economic concern in ND; GWLB. The objectives of the second study were to evaluate phenotypic and genotypic differences among isolates of the ND *C. nebraskensis* population. Results indicate significant differences in the amount of disease caused and the rate of disease progression by the isolates. Genetic differences among isolates also exist, but both phenotypic and genotypic differences appear to be random with no association to isolate origin. The objective of the third study was to evaluate yield loss due to GWLB based on infection timing and hybrid resistance. Results indicated that infection by *C. nebraskensis* at vegetative growth stages caused yield loss in excess of 40% on the susceptible hybrid, while infection at silking resulted in losses around 2%. Regardless of infection timing, yield loss on the resistant hybrid did not exceed 11%. The impacts of these studies will be to direct research efforts to

economically important diseases (GWLB) and to strengthen GWLB management

recommendations for ND corn growers.

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To Caitlin: thank you for giving me the push to pursue graduate school and for supporting me the entire way through.

To Mitch: thank you for making this journey with me. I am grateful for every moment with you.

DEDICATION

I would like to dedicate this dissertation to my parents, Dan and Brenda. Their sacrifices,

perseverance, love, care, and commitment have gotten me to where I am today. I will be forever

grateful.

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LITERATURE REVIEW

Introduction

Goss's wilt and leaf blight, caused by the bacterium *Clavibacter nebraskensis* (Vidaver and Mandel 1974) Li et al. 2018, was first described in Dawson County, Nebraska in 1969. The disease was first called leaf freckles and wilt of corn as a reflection of the characteristic symptoms of each phase of the disease (Schuster 1975). In 1969, the disease was found to be present in three fields in central Dawson County, and by 1972, the disease was reported in 23 Nebraska counties (Schuster 1975; Wysong et al. 1973). Over the next 10 years, the pathogen spread to neighboring states including Colorado, Iowa, Kansas, and South Dakota (Vidaver et al. 1981). The rise in disease prompted corn breeding efforts to focus on developing hybrids with acceptable levels of resistance to the disease.

Reports of yield loss due to Goss's wilt and leaf blight were minimal throughout the late 1980s and 1990s, aside from instances in susceptible sweet corn and popcorn fields. Moreover, reports of the disease were very few after the introduction of resistant hybrids. Over the next two decades, the focus of breeding programs transitioned to yield and quality rather than screening for resistance to Goss's wilt and leaf blight. In 2006, almost 30 years after the initial report, an epidemic of Goss's wilt and leaf blight occurred in Nebraska and the central high plains (Jackson et al. 2007b). Disease reports extended throughout the Corn Belt, the southern United States, and as far north as Canada. As of 2018, the disease has been confirmed in Colorado, Illinois, Indiana, Iowa, Kansas, Louisiana, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota, Texas, Wisconsin, Wyoming, and in Alberta and Manitoba in Canada (Desjardins 2010; Friskop et al. 2014; Howard et al. 2015; Jackson-Ziems et al. 2012; Korus et al. 2011; Malvick et al. 2010; Ruhl et al. 2009; Singh et al. 2015; Sweets and Hosack 2014).

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Corn Production and Corn Diseases in North Dakota

Field corn (*Zea mays* L.) is produced in North Dakota (ND) for grain and silage. Grain corn in North Dakota is grown for industrial use, processing, or for biofuel (i.e. ethanol) production. In recent years, the areas planted to silage corn have been decreasing, while grain corn production has increased in ND (Ransom et al. 2004). Reasons for increased grain corn production include better yielding hybrids, suitable environments (weather patterns have provided more moisture during periods of high water use for corn), and high market prices. Corn production in ND accounted for 3.1% of the U.S.' 2017 grain corn total, with ND ranking 11th among corn producing states (NASS 2017). In hectarage, ND planted 1.4 million hectares for grain production in 2017, while the total U.S. hectarage of grain corn was 36.5 million hectares. Corn production in ND has nearly doubled since 2011. Hectarage has increased from 890,000 hectares planted for grain in 2011 to 1.4 million hectares in 2017 (NASS 2107). In eastern ND, corn is grown in rotation with crops such as wheat, soybean, dry edible bean, and sugarbeet often in conventional tillage systems. In areas further west, corn is more commonly grown for silage and is grown in rotation with crops such as wheat, soybean, and sunflower and in a no- to minimum-tillage system. Rotation lengths can vary by region depending on disease pressure and market prices of the other crops included in the rotation (A. Friskop, personal communication).

In 2009, the first formal corn disease survey was conducted in the state (Ransom et al. 2016). After a cool wet fall, corn fields were surveyed to determine the presence of ear molds and to identify any mycotoxin contamination. Fungal contaminants on corn ears were identified and included *Cladosporium*, *Fusarium*, *Alternaria*, *Penicillium*, *Aureobsidium*, *Rhizopus*, and *Stemphylium* species. However, mycotoxin contamination was low leading researchers to conclude that even if conditions are favorable for ear mold development, mycotoxin

contamination is unlikely. Subsequent years had unfavorable weather conditions for ear mold

development, and so, the survey was not continued. No reports of a formal foliar disease survey

on corn in ND are available.

Pathogen Biology

      Clavibacter nebraskensis is a rod-shaped, non-motile, gram positive bacterium belonging

to the class *Actinobacteria* and family *Microbacteriaceae* (Stackebrandt et al. 2007). On semi-

selective media, either nutrient broth yeast (NBY) or *Corynebacterium nebraskense* selective

(CNS), *C. nebraskensis* produces characteristic apricot-orange, round, mucoidal colonies (Gross

and Vidaver 1979). *Clavibacter nebraskensis*, was originally known as *Corynebacterium*

nebraskense (Vidaver and Mandel 1974). In 1982, *Corynebacterium nebraskense* became

Corynebacterium michiganse subsp. *nebraskense* after Carlson and Vidaver proposed to classify

C. michiganense, C. *nebraskense*, *C. insidiosum*, *C. sepedonicum*, and *C. tessellarius* under a

single species. Polyacrylamide gel electrophoresis of cellular proteins showed high similarity

between the nomenspecies, which provided the basis for a single species classification. Because

of differences in colony morphology, pigmentation, and bacteriocin production, the

nomenspecies were classified as subspecies of *C. michiganense* (Carlson and Vidaver 1982). The

reclassification of the genus *Corynebacterium* to *Clavibacter* occurred in 1984 on the basis of

cell wall composition (Davis et al. 1984). The subspecies descriptions were retained under the

name *Clavibacter michiganensis*. All five subspecies (*michiganensis*, *nebraskensis*, *insidiosus*,

sepedonicus, and *tessellarius*) were host-specific (tomato, maize, alfalfa, potato, and wheat,

respectively) and transmissible by seed. Four additional subspecies of *Clavibacter michiganensis*

have since been described (Gonzalez and Trapiello 2014; Oh et al. 2016; Yasuhara-Bell and

Alvarez 2015). These include disease-causing bacteria on bean, pepper and tomato: bacterial leaf

yellowing on bean caused by *C. michiganensis* subsp. *phaseoli*, bacterial canker of pepper caused by *C. michiganensis* subsp. *capsici*, and *C. michiganensis* subsp. *californiensis* and *C. michiganensis* subsp. *chilensis* which were isolated from tomato and pepper seeds from California and Chile, respectively (Gonzalez and Trapiello 2014; Oh et al. 2016; Yasuhara-Bell and Alvarez 2015).

The elevation of *C. michiganensis* subspecies to the species level has been proposed (Li et al. 2018). Whole-genome sequencing and a multi-locus sequence analysis (MLSA) were conducted using two strains each of *C. michiganensis* subsp. *sepedonicus* and *C. michiganensis* subsp. *tessellarius*, six strains of *C. michiganensis* subsp. *nebraskensis*, and the type strains of *C. michiganensis* subsp. *michiganensis*, *C. michiganensis* subsp. *insidiosus*, and *C. michiganensis* subsp. *tessellarius*. Genome sequences of each strain obtained in the study were compared to those available in GenBank. Average nucleotide identity (ANI) and digital DNA-DNA hybridization (dDDH) thresholds were used to determine species delineation. The values of ANI represent the level of similarity between homologous regions shared by two genomes. The values of dDDH represent the distance between pairs of genomes that are either partially or fully sequenced. For species delineation, ANI values must be below the 96% cutoff value, while dDDH values must be below 70%. The range of ANI values among the *Clavibacter* subspecies sequenced was from 89 to 95%. The corresponding dDDH values were between 37 and 60% (Li et al. 2018). Taxonomic relationships were resolved using MLSA on sections of housekeeping genes from closely related species. The five *Clavibacter* subspecies fell into five distinguishable clusters. The five clusters matched the five genomospecies provided by ANI and dDDH analysis (Li et al. 2018). The preceding evidence led Li et al. to propose the re-classification of *Clavibacter* subspecies. Therefore, *Clavibacter michiganensis* subsp. *nebraskensis* was proposed

to be a new combination at the species level: *Clavibacter nebraskensis* comb. nov (Li et al. 2018). From this point forward in this document, what was once referred to as *Corynebacterium nebraskense*, *Clavibacter michiganense* subsp. *nebraskense*, and *Clavibacter michiganensis* subsp. *nebraskensis* will be called *Clavibacter nebraskensis*.

<u>Disease Epidemiology</u>

Clavibacter nebraskensis predominately requires a wound to enter and infect host tissue. Wounding events such as wind, rain, sand blasting, and mechanical damage from farm equipment most often provide such entry points. Natural openings, such as stomata or the base of trichomes, can also serve as entry points for *C. nebraskensis* (Mallowa et al. 2016). Once established in the host, *C. nebraskensis* spreads through plants' xylem tissue. Although all former subspecies of *C. michiganensis* are seed-transmissible, it was found that *C. nebraskensis* seed transmission occurs at a very low rate, 0.1 to 0.4% (Biddle et al. 1990). Two disease phases are associated with *C. nebraskensis* infection: a foliar blight phase and a systemic wilt phase. Necrotic lesions of the leaf blight phase kill leaf tissue reducing photosynthetic areas, while the wilt phase causes drought stress to plants (Jackson et al. 2007a; Wise et al. 2010). On leaf surfaces, *C. nebraskensis* produces an extra-polysaccharide exudate that can ooze out of infected leaf tissue. The exudate dries on leaf surfaces on exposure to sunlight giving infected leaves a shiny or glossy-like appearance. Bacterial colonization of stems during systemic infection results in the plugging of xylem tissue and can be identified by orange discoloration that may turn black and be slimy as the infection progresses (Jackson et al. 2007a). *Clavibacter nebraskensis* can survive in infected leaf tissue or on leaf surfaces as a dried exudate for up to 10 months in the field (Schuster 1975; Smidt and Vidaver 1986). Infected debris can serve as initial inoculum for the successive corn crop. Infection is favored by warm weather (26 to 32°C), with 27°C as the

optimal growth temperature (Smidt and Vidaver 1986). Relative humidity (RH) also impacts

bacterial survival and infection rates. Mallowa et al. (2016) observed an increase in population

densities of epiphytic *C. nebraskensis* at high RH (20 to 40% higher than ambient RH), while

Leben (1988) also reported increased epiphytic survival and colonization by *Pseudomonas*

syringae pathovars on cucumber plants at high RH (80 to 90%). However, warm and dry

conditions can limit the development of Goss's wilt and leaf blight (Jackson et al 2007a).

Frequently, the onset of infection is seen following severe summer storms. Wind and water

droplets can disseminate inoculum within and between corn fields. Wind can deposit infected

debris in healthy fields as well as transfer epiphytic populations of the bacteria from leaf-to-leaf

as leaves rub together in the wind. Aerosols provide a possible explanation for how bacteria

spread over long distances (Graham and Harrison 1975; Venette and Kennedy 1975).

Clavibacter nebraskensis can survive epiphytically on corn leaves while the leaves remain

asymptomatic, and it is possible that populations build up gradually over time in new areas

before disease is detected (Eggenberger et al. 2016). Overwintering bacteria on debris can be

splashed by rain or irrigation water onto leaves of new corn plants. Thus, infection may first

occur in the lower canopy then progress upwards through further water splash of bacteria or

through xylem tissue before spreading systemically.

Corn is the primary host of *C. nebraskensis*. Field (yellow dent) corn, sweet corn, and

popcorn are all susceptible to the pathogen with sweet corn and popcorn being more susceptible

than field corn. Several alternative weed hosts have been identified, which may serve as

inoculum reservoirs. An original report of alternative hosts of *C. nebraskensis* included teosinte

(*Zea mexicana*), eastern gamma grass (*Tripsacum dactyloides*), green foxtail (*Setaria viridis*),

shattercane (*Sorghum bicolor* subsp. *arundinaceum*), grain sorghum (*Sorghum bicolor*),

sudangrass (*Sorghum bicolor* subsp. *drummondii*), and sugarcane (*Saccharum officinarum*) (Schuster 1975). Although barnyardgrass (*Echinochloa crus-galli*) was later reported as an alternative host (Wysong et al. 1981), Schuster (1975) along with Ikley et al. (2015) found barnyardgrass to be a non-host. *Clavibacter nebraskensis* was found epiphytically on leaves of barnyardgrass, but was unable to infect the plant (Ikley et al. 2015). More recent studies have identified new and and/or confirmed original reports of weed hosts of *C. nebraskensis*. Recently reported weed hosts include giant foxtail (*Setaria faberi*), bristly foxtail (*Setaria verticillata*), yellow foxtail (*Setaria pumila*), annual ryegrass (*Lolium multiflorum*), johnsongrass (*Sorghum halepense*), and large crabgrass (*Digitaria sanguinalis*) (Ikley et al. 2015; Langemeir et al. 2014).

Symptoms and Signs

Goss's leaf blight is recognizable by necrotic lesions with wavy margins that can extend the length of the leaf along veins. The first symptom to appear in the leaf blight phase is water soaking. The characteristic symptom of Goss's leaf blight, "freckling", can be seen within water-soaked areas. Water soaking can appear irregular or discontinuous on leaves. Freckles result from these discontinuations in water soaking and may appear dark green to black and will not rub off of the leaf (Jackson et al. 2007a). As bacterial exudate oozes out of leaves, it can dry on leaf surfaces leaving a shiny appearance of the lesion. With severe foliar infections, large necrotic areas may be confused with drought stress. Goss's wilt can be recognized by systemically infected plants. In this phase of the disease, the bacteria move through the plant via xylem tissue. Infected xylem tissue will be discolored, turning apricot-orange to brown or black and will often ooze out of vascular bundles giving the infection a slimy appearance. Eventually,

the systemic infection will cause plants to wilt and die, especially in the case of young seedlings (Jackson et al. 2007a).

Yield Loss Caused by Goss's Wilt and Leaf Blight

Goss's wilt and leaf blight was estimated to be in the top-ten most destructive diseases of corn in the northern U.S. and Ontario, Canada from 2012 to 2015 (Mueller et al. 2016). In 2013, 2014, and 2015, Goss's wilt and leaf blight was estimated to be in the top-four most destructive diseases, only consistently outranked by northern corn leaf blight. Yield loss attributed to Goss's wilt and leaf blight has been assessed using both observational data and field research data. It was estimated that from 2012 to 2015, yield losses in the U.S. and Ontario, Canada due to corn disease exceeded 155 billion kg (Mueller et al. 2016). Yield losses during the same four years due to Goss's wilt and leaf blight alone were estimated to be approximately 13 billion kg. In financial terms, corn diseases caused an estimated loss of $27.4 billion in the U.S. and Ontario, Canada from 2012 to 2015. In ND alone, an estimated $100 million were lost due to corn disease in the same four-year span (Mueller et al. 2016).

Under high levels of disease, yield losses as high as 3,700 kg/ha were reported in northwest Indiana (Wise et al. 2010), losses of 30% have been reported in Minnesota (Malvick 2018), and the use of very susceptible hybrids resulted in yield losses exceeding 50% (Claflin 1999). Inoculated field trials reported yield losses of 55% on susceptible hybrids (Malvick et al. 2014) and losses as high as 44% on susceptible inbred lines (Carson and Wicks, 1991). Although these reports have documented the importance of the disease on susceptible hybrids and inbred lines, the extent of yield loss will vary depending on the level of host resistance and disease onset. Calub et al. (1974) indicated inoculation timings completed on two-week-old seedlings routinely resulted in higher disease severity than inoculations on four-, six-, or eight-week-old

seedlings. Resistant crosses had significantly less disease when inoculated after eight weeks of growth than did susceptible material. Additionally, disease ratings on resistant material decreased as age at inoculation increased. Inoculation timings on susceptible sweet corn indicated disease severity was highest and yield was lowest when inoculated at the three-to-five leaf stage (Suparyono and Pataky 1989). However, when a resistant hybrid was used, inoculation timing had very little impact on either disease severity or yield.

Diversity of *Clavibacter nebraskensis*

Limited studies on variation or genetic diversity within or between *C. nebraskensis* populations have been conducted. Bacteriophage and bacteriocin typing were used to group 85 *C. nebraskensis* strains collected between 1969 and 1979 (Vidaver et al. 1981). Although both methods grouped the strains into eight groups, no correlation was found between groups and either the year of isolation or the geographic origin of the strain. Consequently, the *C. nebraskensis* population was not found to be variable at that time point. A morphology study observed differences in *C. nebraskensis* colonies (strains) isolated in 1982 from one popcorn field (Smidt and Vidaver 1987). Fifty strains were isolated from both plants and plant debris and based on colony morphology, pathogenicity, and toxin production, the strains clustered into seven groups. Population diversity was also identified by bacteriophage sensitivity, as the seven groups of *C. nebraskensis* were further divided into 20 distinct groups. However, no correlation was found between which strains belonged to a group and whether the strains were isolated from a plant or debris, or where the plant or debris was located within the field (Smidt and Vidaver 1987).

Genetic diversity has been examined among *C. nebraskensis* isolates using molecular techniques. Agarkova et al. (2011) analyzed 131 isolates collected from 1969 to 2009 using both

amplified fragment length polymorphism (AFLP) analysis and repetitive DNA sequence-based BOX-PCR. The isolates were shown to cluster into two groups; 118 isolates in group A and 13 isolates in group B. A composite analysis of data from both the AFLP analysis and BOX-PCR showed that the genome of Group A had been stable for a long period of time. The 13 strains in group B represented recent genetic changes between 1999 and 2009. No correlation between origin, history, morphology, or physiology (defined by results of Gram staining and KOH testing, *C. nebraskensis* is gram positive and negative for the KOH test) for the isolates in group A (Agarkova et al. 2011).

A study comparing *C. nebraskensis* isolates isolated from symptomatic and asymptomatic corn leaves found differences in aggressiveness, in terms of proportion of leaf area infected, among isolates (Ahmad et al. 2015). However, no relationship existed between aggressiveness and the origin of the isolate (i.e. from asymptomatic or symptomatic leaf tissue). In a preliminary step to identify *C. nebraskensis* genes involved in virulence, the study examined 33 candidate virulence *C. nebraskensis* genes in both groups of isolates. When pathogenic and non-pathogenic *C. nebraskensis* isolates were compared, sequence polymorphisms were found in 5 genes: cellulose A, two endoglucanases, xylanase B, and a pectate lyase. However, no relationship was found between polymorphisms present and the pathogenicity of the isolates (Ahmad et al. 2015).

Several groups have investigated genetic variability among and between populations of other *Clavibacter* species. Multilocus sequence analysis (MLSA) and repetitive sequence-based (rep-PCR) genomic fingerprinting documented introductions and the spread of *C. michiganensis* subsp. *michiganensis* in Turkey and Argentina, respectively (Sen et al. 2018; Wassermann et al. 2017). In Turkey, 108 *C. michiganensis* subsp. *michiganensis* strains collected over a 16-year

span were found to be genetically similar (Sen et al. 2018). The genetic uniformity within the population supported the idea of an initial introduction and rapid dissemination of *C. michiganensis* subsp. *michiganensis* in Turkey. Furthermore, the consistency of the Turkish *C. michiganensis* subsp. *michiganensis* population indicates that its spread through the country is most likely do to agronomic practices. Multilocus sequence typing (MSLT) analysis of *C. michiganensis* subsp. *michiganensis* housekeeping genes revealed that the majority of the strains fell into a single group that was widespread throughout Turkey. Only strains that were isolated after 2004 fell into other groups, indicating few other isolated introductions of *C. michiganensis* subsp. *michiganensis* into Turkey (Sen et al. 2018).

A genetic analysis of *C. michiganensis* subsp. *michiganensis* in Argentina indicated new introductions occur every year (Wasserman et al. 2017). A total of 12 *C. michiganensis* subsp. *michiganensis* strains collected from 5 greenhouse locations over a span of 14 years were found to be genetically diverse. The 12 strains fell into 3 distinct groups, although the grouping was not associated spatially or temporally. Strains from multiple groups were present in each greenhouse, indicating multiple sources of inoculum or multiple introductions of *C. michiganensis* subsp. *michiganensis*. Likely, *C. michiganensis* subsp. *michiganensis* is being re-introduced into Argentina each year via infected tomato seed lots (Wasserman et al. 2017).

Recently, a robust study utilized 16S rRNA and genome-based DNA homology to evaluate the taxonomic position of former *Clavibacter* subspecies (Tambong 2017). The evidence reported in Tambong's (2017) study was cited as further support of the decision to elevate some *Clavibacter* subspecies to the species level (Li et al. 2018). The genomes of former *Clavibacter* subspecies were analyzed providing details into the functional organization of each. At 3.06 Mbp, *C. nebraskensis* has the smallest genome and unlike other *Clavibacter* species, *C.*

nebraskensis does not contain plasmids (Tambong 2017). It is believed that plasmids are not required for virulence in *C. nebraskensis* (Gross et al. 1979). Genes encoded in the plasmids of other *Clavibacter* species are likely chromosomally encoded in *C. nebraskensis* (Tambong 2017). Furthermore, it is implied that because *C. nebraskensis* has the smallest genome, it also has the fewest protein-encoding genes (Tambong 2017). Therefore, it is thought that *C. nebraskensis* requires only a small number of genes for survival in host tissue (Tambong 2017). However, the virulence strategies of *C. nebraskensis* are still relatively unknown (Ahmad et al. 2015).

Management and Strategies

Proper identification of a disease and its causal agent is necessary before management decisions are considered. Serological techniques such as enzyme-linked immunosorbent assays (ELISA) have been used to identify *C. nebraskensis* (Korus 2011). In the field, Agdia ImmunoStrip kits, an ELISA test, have been used for quick identification of the pathogen. Although not specific to *C. nebraskensis*, the bacterium can cross react with any *C. michiganensis* antibodies present, giving a positive result (Korus 2011). Recently though, several specific and reliable molecular techniques have been developed for the identification of *C. nebraskensis*. Loop-mediated amplification (LAMP), novel gene targets for use in both conventional and quantitative polymerase chain reaction (PCR), and multiplex TaqMan real-time PCR are available for rapid and accurate detection of *C. nebraskensis* (McNally et al. 2016; Tambong et al. 2016; Yasuhara-Bell et al. 2016).

There are several options for managing Goss's wilt and leaf blight. However, an integrated approach including cultural practices and genetic resistance is most effective. Rotating away from corn for at least one year and incorporating debris into soil to promote decomposition

of residue and reduce bacterial survival will reduce primary inoculum sources for the subsequent corn crop. Any type of tillage, such as plowing or even disking, are sufficient methods to bury debris and promote decomposition of bacteria (Eggenberger et al. 2016). Genetically resistant hybrids are available and are an excellent management strategy for Goss's wilt and leaf blight. The idea that resistance to Goss's wilt and leaf blight is inherited in a polygenic manner has been supported by multiple studies. While breeding for Goss's wilt and leaf blight resistance in early-maturing lines, it was determined that resistance to the disease is quantitative and controlled by only a few genes (Ngong-Nassah et al. 1992). High general combining ability suggests that the resistance genes work in an additive manner (Treat et al. 1990). The first study to use linkage mapping for identification of quantitative trait loci (QTLs) responsible for resistance to Goss's wilt and leaf blight, also supported polygenic inheritance. While 11 QTLs were identified with mapping techniques, only a small amount of phenotypic variation could be explained by each (Singh et al. 2016).

Physiological components of resistance to Goss's wilt and leaf blight have also been studied (Mbofung et al. 2016). Scanning electron microscopy was used to examine the composition of corn leaves of both resistant and susceptible hybrids that were artificially inoculated with *C. nebraskensis*. In infected tissue, it was documented that *C. nebraskensis* cells were misshapen and that xylem tissue contained a dense matrix, likely to restrict further movement of *C. nebraskensis* through the plant (Mbofung et al. 2016). It was suggested that resistant hybrids are likely able to deploy an additional mechanical or chemical defense to confine *C. nebraskensis* below a harmful titer. To note a concern, Jackson et al. (2007b) reported that less than 25% of seed companies rated their hybrids for resistance to Goss's wilt and leaf blight. The lack of information available on hybrid resistance is a possible explanation of how

susceptible hybrids were widely distributed at the time of the disease's re-emergence in 2006 (Jackson et al. 2007b). Since then, hybrid resistance ratings to Goss's wilt and leaf blight are regularly included.

The use of chemical control products have been evaluated to observe their value in reducing Goss's wilt and leaf blight severity. Recent work evaluated the efficacy of copper hydroxide and citric acid in reducing the severity of Goss's wilt and leaf blight (Mehl et al. 2015). Corn plots were inoculated with *C. nebraskensis* and treatments consisted of application of either copper hydroxide or citric acid at a timing either prior to or following inoculation. Although non-inoculated plots had significantly lower disease severity than did inoculated plots, chemical treatment did not have a significant impact on reducing disease within inoculated or non-inoculated plots. Furthermore, neither inoculation nor chemical treatment significantly impacted yield and the authors concluded that copper hydroxide and citric acid are not viable management options for Goss's wilt and leaf blight (Mehl et al. 2015). Likewise, other studies have evaluated chemical efficacy and timing for the management of Goss's wilt and leaf blight. Application of a copper hydroxide the day after inoculation resulted in yield that was significantly greater than an application of an industry standard fungicide (Korus et al. 2010). However, the yield of these treatments was not significantly different than the non-treated inoculated check. In a trial conducted under very warm temperatures, yields were not significantly different between treated and non-treated plots regardless of treatment type (fungicide, bactericide, or both) and application timing (prior to inoculation, after inoculation, at symptom onset) (Oser et al. 2013). In an additional trial conducted under warm and dry conditions, neither treatment type nor application timing significantly affected disease severity,

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even in inoculated plots (Wise et al. 2014). The results of these studies indicate that chemical control is not recommended for management of this disease.

With an increase in corn production in ND and the risk of yield loss associated with disease, there is a need to investigate the prevalence, diversity, and management of Goss's wilt and leaf blight and its causal agent *C. nebraskensis* in the state. The objectives of this study will address the most commonly asked questions in ND regarding the pathogen and management of the disease.

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CHAPTER 1. PREVALENCE OF CORN FOLIAR DISEASES IN NORTH DAKOTA

<u>Introduction</u>

Corn production in North Dakota (ND) has nearly doubled since 2011. Hectarage has increased from 890,000 hectares planted for grain in 2011 to 1.4 million hectares in 2017 (NASS 2017). Corn production in ND accounted for 3.1% of the United States' 2017 grain corn total, with ND ranking 11th among corn producing states (NASS 2017). In hectarage, ND planted 1.4 million hectares for grain in 2017, while the total U.S. hectarage of corn for grain was 36.5 million hectares. Typically, the majority of corn production occurs in the East Central, Central, and Southeast districts of the state. In 2017, the southeast quarter of the state was responsible for 62% of ND's corn production. With favorable grain prices, corn hectarage has increased in several areas of ND, yet the prevalence of foliar corn disease has not been documented.

Corn yield loss estimates due to disease from 2012 to 2015 were reported for corn producing states in the U.S. and Ontario, Canada (Mueller et al. 2016). Yield losses from corn diseases were estimated to be 10.9%, 7.5%, 10.4%, and 13.5% for 2012, 2013, 2014, and 2015, respectively. These reports are similar to earlier estimates of corn disease losses, which were approximated at 2 to 15% annually (White and Carson 1999). North Dakota's estimated yield losses during the survey years were 116 million kg, 105 million kg, 252 million kg, and 170 million kg in 2012, 2013, 2014, and 2015, respectively. Yield losses in ND caused by foliar diseases alone were 111 million kg, 103 million kg, 167 million kg, and 85 million kg in 2012, 2013, 2014, and 2015, respectively (Mueller et al. 2016). The associated yield losses with corn diseases in ND prompt further investigation into documenting the prevalence of diseases in the state.

Surveys are a useful tool for identifying the presence and/or prevalence of plant diseases. Surveys have been used to estimate economic losses, determine prevalence and incidence of disease, determine factors contributing to disease, determine the presence of mycotoxins, and identify races of a pathogen present in a region (Gulya et al. 2013; Langemeier et al. 2017; Mueller et al. 2016; Ransom et al. 2016; Weems and Bradley 2018). In ND, very few surveys have been conducted on corn. In 2009, Ransom et al. conducted a late-season ear mold survey to obtain information on disease prevalence and analyzed associated mycotoxin production (Ransom et al. 2016). Unseasonably cool and wet weather in October of 2009 created optimal conditions for corn ear mold development in ND. Because of concern for mycotoxin contamination, corn ear samples from 94 fields in 24 counties were examined macroscopically and microscopically to identify fungal contaminants. The fungal species most frequently identified were *Cladosporium*, *Fusarium*, *Alternaria*, and *Penicillium* (Ransom et al. 2016). Mycotoxins were found in only 27% of the samples. Deoxynivalenol, T-2, HT-2, zearalenone, and nivalenol were the toxins identified, but most toxin levels were below 2 ppm. In samples where deoxynivalenol was confirmed, 73% had levels below 1 ppm. Importantly, the survey results indicated that ear mold problems were primarily caused by saprophytes with marginal concerns for mycotoxins.

Field surveys have already been used in ND to document disease prevalence on an important disease of sunflower. A multi-year survey on downy mildew prevalence (percentage of infected fields) was completed (Gulya et al. 2013). Across the 10 years of the survey, greater incidence and prevalence were recorded between late May and mid-August (mid-season) than from mid-September to mid-October (late-season). The sunflower downy mildew study successfully used field survey techniques and environmental data to explain prevalence levels of

an endemic disease in ND. This survey also provided valuable insight on the importance of downy mildew and the incorporation of an integrated management strategy for the disease.

In 2011, the first report of Goss's wilt and leaf blight was documented in Emmons County in south central ND. The yield-limiting disease of corn is caused by the gram positive bacterium *Clavibacter nebraskensis* (Vidaver and Mandel 1974) Li et al. 2018. Yield losses from Goss's wilt and leaf blight can be severe in a given year and depend on several factors including timing of disease onset and hybrid susceptibility. The bacterium can overwinter for up to 10 months in infested residue on the soil surface (Schuster 1975; Smidt and Vidaver 1986). As is common for most plant pathogenic bacteria, *C. nebraskensis* enters host tissue through wounds or natural openings (Mallowa et al. 2016). Infection may occur in localized pockets within fields or under optimal conditions (i.e. overhead irrigation), may be widespread in a field.

To our knowledge, a formal foliar disease survey of corn has not been previously conducted in ND. The objectives of this study were to document the prevalence of foliar diseases of corn in ND; determine disease prevalence differences among ND counties and years; and to evaluate relationships of weather data on foliar disease prevalence.

Materials and Methods

Sampling

Beginning in 2014 and continuing annually through 2018, corn fields in ND were arbitrarily selected and scouted for foliar diseases. Counties with historically high corn acreage were visited more frequently (Figure 1.1). Scouting was conducted between the months of July and September, when corn was between the reproductive growth stages R1 (silk) and R5 (dent). In each field, GPS coordinates were obtained, and 100 plants were examined in a "W" pattern with 20 plants at 5 points along transects of the "W". Diseases were recorded based on foliar

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symptoms and/or pathogen signs. Symptomatic leaves that could not be diagnosed visually in the field were brought back to the laboratory for further examination. Leaves exhibiting Goss's leaf blight symptoms were collected and brought to the laboratory for isolation of the pathogen. In 2014, scouting was conducted by members of the NDSU Cereal Extension Plant Pathology Program (NCEPPP). In 2015 and 2016, NDSU Extension Plant Pathology scouts were hired to assist in the corn survey. With the assistance of the crop scouts, corn fields were visited in the western two-thirds of the state. The scouting protocol written in 2015 and 2016 did not require scouts to record incidence of common smut. Therefore, data were not uniformly recorded for this disease. In 2017 and 2018, members of the NCEPPP reported on all foliar diseases and some ear and stalk diseases.
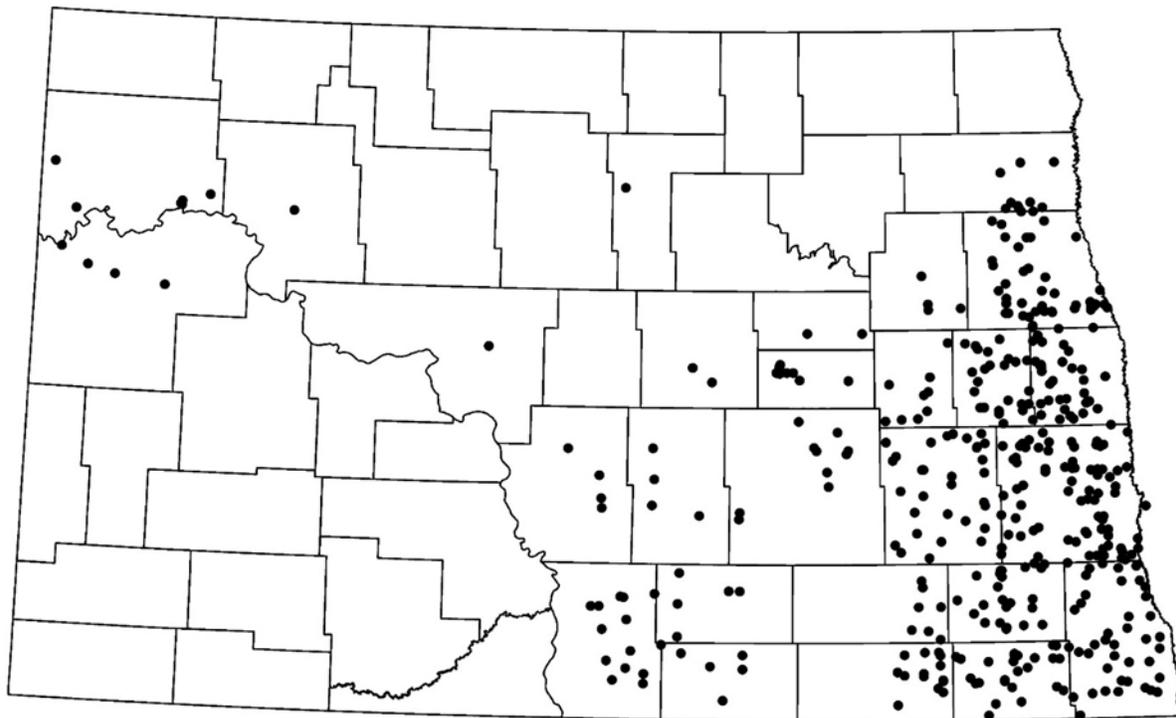
Figure 1.1. Distribution of surveyed fields during the 2014 to 2018 North Dakota corn foliar disease surveys. Each (●) represents a surveyed field.

Disease identification

Foliar symptoms and pathogen signs were used to identify corn diseases in the field. Common rust (CR) was identified by the brick-red rust pustules of *Puccinia sorghi* present on both the adaxial and abaxial leaf surfaces (Jackson-Ziems 2014; Wise 2010). Individual pustules can be round or elongated. As rust pustules form, they break through the epidermal layer of the leaf. The disease was quickly identifiable in the field as rust pustules can be easily rubbed off of leaf surfaces. Common smut (CS) caused by *Ustilago maydis* produces easily identifiable galls on any meristematic tissue, but most commonly on stalks, ears, leaves, and tassels (Pataky and Snetselaar 2006). Newly formed galls are white in color. As galls mature, black streaks become evident in the tissue as the galls are filled with powdery black teliospores. Northern corn leaf blight (NCLB) was identified by tan, elliptical to cigar-shaped necrotic lesions with well-defined margins. Lesions ran parallel to leaf veins and were not limited to veins. Upon close inspection, black conidia of the fungus (*Exserohilum turcicum*; teleomorph *Setosphaeria turcica*), can be seen in the center of disease lesions when periods of high humidity occur (Wise 2011). The symptoms of NCLB can differ depending on hybrid. With resistant hybrids, lesions may appear smaller than on susceptible hybrids and sporulation may not occur (Jackson-Ziems 2015). When symptoms were inconclusive in the field, suspect-NCLB leaves were brought to the laboratory and placed in humidity chambers to induce sporulation. After 24 h, necrotic areas were examined microscopically for sporulation. Goss's leaf blight (GLB) was visually identified by wavy, chlorotic to necrotic lesions surrounded by water soaking that could extend the length of corn leaves and ran parallel to the mid-rib. Dark green to black freckles appear in water-soaked areas and do not rub off of the leaf (Jackson et al. 2007). When held to the light, the freckled areas are translucent. Additionally, the dried bacterial exudate (extra-polysaccharide) can give the leaf

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surface a shiny appearance. Leaves with inconclusive symptoms were again brought back to the laboratory for further examination. Symptomatic areas were observed microscopically for bacterial streaming, and infected leaf tissue was cultured on nutrient broth yeast (NBY) or *Corynebacterium nebraskense* selective (CNS) media. Bacterial streaming and/or the presence of round, mucoid, apricot-orange colonies confirmed samples to be that of GLB. Agdia Immunostrips were also used to positively identify samples.

Weather data

The North Dakota Agricultural Weather Network (NDAWN) records weather parameters such as temperature, wind speed, soil moisture, and rainfall at stations throughout the state. Data were compiled for the five survey years from NDAWN's online database. Data were acquired from all stations within the counties encompassed by the five-year survey. If multiple weather stations were located within a single county, weather data were averaged across the station locations to provide one county mean. For each survey year, average monthly temperature (°C), total monthly rainfall (mm), and departures from normal (the 30-year average) were acquired from each station for the months of May through August to represent weather during the corn growing season of ND. Rainfall and average temperature were also acquired for three time periods, from May 15 to June 15, June 15 to July 15, and July 15 to August 15 of each year. Temperature and moisture were selected as the variables to report due to their influence on GLB development or lack thereof. Weather data were used for correlation analysis with GLB prevalence.

Data analysis

For this study, disease prevalence is defined as the number of infected fields divided by the total number of fields surveyed in a county or year and multiplied by 100. A chi-square

analysis was performed at the 99% level of confidence to determine if significant differences in disease prevalence occurred among survey years, counties, or diseases. A least squares means post-hoc analysis with a Tukey-Kramer adjustment was done to statistically separate prevalence differences among years, counties, or diseases at the 95% level of confidence. Linear relationships between rainfall or average temperature and GLB prevalence across counties were tested using PROC CORR in Statistical Analysis Software (SAS) (v. 9.4, SAS Institute, Cary, NC). Pearson correlation coefficients were used to observe strengths of relationships at three time periods (May 15 to June 15, June 15 to July 15, and July 15 to August 15) for each year.

Results

Four diseases were commonly reported across the five survey years. These included CR, CS, NCLB, and GLB. The wilt phase of Goss's wilt and leaf blight was infrequently detected in ND (less than ten fields across five survey years). Therefore, in this document, the disease is referred to as Goss's leaf blight (GLB), the more common disease phase in ND. Holcus leaf spot, Fusarium ear mold, and Fusarium stalk rot were also documented in the surveying efforts, but due to infrequent observations, prevalence data is not reported for these diseases. In 2014, a total of 57 corn fields in 14 counties were visited. Foliar diseases recorded included CR, CS, NCLB, and GLB. The overall prevalence of CR, CS, NCLB, and GLB were 91%, 25%, 37%, and 32%, respectively. In 2015, 80 fields were scouted in 15 counties. The overall prevalence of CS in 2015 was 41%, followed by CR at 40%, NCLB at 19%, and GLB at 6%. In 2016, 82 fields in 11 counties were included in the survey. Disease prevalence in 2016 ranged from 15% for NCLB to 80% for CR. Common smut and GLB were observed in 29% and 24% of fields, respectively. In 2017, 103 fields were scouted in 14 counties. The prevalence of CR, CS, and GLB across surveyed counties were 93%, 44%, and 35%, respectively. Unique to 2017, NCLB was not

documented in ND. The 2018 survey included 109 fields across 23 counties. Common rust, CS, NCLB, and GLB were observed in 41%, 62%, 2%, and 66% of fields, respectively. The breakdown of disease prevalence for each disease across years can be seen in Tables 1.1 to 1.4. The distribution of GLB incidence across years is presented in Figure 1.2.

Table 1.1. Prevalence (%) of common rust in North Dakota counties from 2014 to 2018.

County	2014 Fields	2014 Prevalence[z]	2015 Fields	2015 Prevalence	2016 Fields	2016 Prevalence	2017 Fields	2017 Prevalence	2018 Fields	2018 Prevalence
Barnes	5	100	3	0	11	100	10	100	5	20
Burleigh	0	-	0	-	0	-	0	-	4	0
Cass	13	92	25	56	14	86	22	100	9	78
Dickey	5	100	6	100	0	-	3	100	2	0
Eddy	0	-	0	-	0	-	0	-	3	100
Emmons	3	67	0	-	6	100	3	100	3	0
Foster	2	100	0	-	3	0	3	100	2	100
Grand Forks	5	80	4	25	6	83	15	53	12	0
Griggs	2	100	2	0	1	100	0	-	4	0
Kidder	0	-	0	-	0	-	0	-	4	75
LaMoure	2	100	0	-	0	-	3	100	2	50
Logan	0	-	0	-	0	-	0	-	5	60
McIntosh	0	-	0	-	0	-	0	-	5	40
McKenzie	0	-	4	0	0	-	0	-	0	-
McLean	0	-	1	0	0	-	0	-	0	-
Mountrail	0	-	1	0	0	-	0	-	0	-
Nelson	0	-	0	-	0	-	0	-	4	0
Pierce	0	-	1	0	0	-	0	-	0	-
Ransom	2	100	6	33	6	83	5	100	3	100
Richland	9	78	3	67	10	70	14	100	5	100
Sargent	1	100	6	50	11	64	5	100	4	100
Steele	1	100	2	0	6	83	8	100	11	27
Stutsman	2	100	0	-	0	-	3	100	5	40
Traill	5	100	11	36	8	88	5	100	5	20
Walsh	0	-	0	-	0	-	4	100	8	25
Wells	0	-	0	-	0	-	0	-	3	67
Williams	0	-	5	0	0	-	0	-	1	100
Total	57	91	80	40	82	80	103	93	109	41

[z]Prevalence defined as the number of infected fields divided by the total number of fields surveyed in a county or year and multiplied by 100.

Table 1.2. Prevalence (%) of common smut in North Dakota counties from 2014 to 2018.

County	2014 Fields	2014 Prevalence[y]	2015 Fields	2015 Prevalence	2016 Fields	2016 Prevalence	2017 Fields	2017 Prevalence	2018 Fields	2018 Prevalence
Barnes	5	20	*[z]	-	11	50	10	40	5	60
Burleigh	0	-	0	-	0	-	0	-	4	100
Cass	13	15	25	57	14	20	22	36	9	78
Dickey	5	40	6	0	0	-	3	100	2	100
Eddy	0	-	0	-	0	-	0	-	3	33
Emmons	3	0	0	-	*	-	3	33	3	0
Foster	2	0	0	-	3	0	3	67	2	100
Grand Forks	5	0	4	0	6	25	15	40	12	50
Griggs	2	100	*	-	*	-	0	-	4	50
Kidder	0	-	0	-	0	-	0	-	4	50
LaMoure	2	50	0	-	0	-	3	67	2	100
Logan	0	-	0	-	0	-	0	-	5	0
McIntosh	0	-	0	-	0	-	0	-	5	40
McKenzie	0	-	*	-	0	-	0	-	0	-
McLean	0	-	*	-	0	-	0	-	0	-
Mountrail	0	-	*	-	0	-	0	-	0	-
Nelson	0	-	0	-	0	-	0	-	4	0
Pierce	0	-	*	-	0	-	0	-	0	-
Ransom	2	0	6	100	*	-	5	40	3	33
Richland	9	33	*	-	10	50	14	57	5	80
Sargent	1	100	6	67	11	0	5	60	4	75
Steele	1	0	*	-	*	-	8	13	11	73
Stutsman	2	0	0	-	0	-	3	0	5	100
Traill	5	40	11	0	8	25	5	80	5	100
Walsh	0	-	0	-	0	-	4	25	8	75
Wells	0	-	0	-	0	-	0	-	3	100
Williams	0	-	*	-	0	-	0	-	1	0
Total	57	25	80	41	82	29	103	44	109	62

[y]Prevalence defined as the number of infected fields divided by the total number of fields surveyed in a county or year and multiplied by 100.
[z]Counties that were not surveyed for common smut are designated with (*).

Table 1.3. Prevalence (%) of northern corn leaf blight in North Dakota counties from 2014 to 2018.

County	2014 Fields	2014 Prevalence[z]	2015 Fields	2015 Prevalence	2016 Fields	2016 Prevalence	2017 Fields	2017 Prevalence	2018 Fields	2018 Prevalence
Barnes	5	0	3	0	11	0	10	0	5	0
Burleigh	0	-	0	-	0	-	0	-	4	0
Cass	13	46	25	32	14	7	22	0	9	0
Dickey	5	60	6	0	0	-	3	0	2	0
Eddy	0	-	0	-	0	-	0	-	3	0
Emmons	3	0	0	-	6	0	3	0	3	0
Foster	2	0	0	-	3	0	3	0	2	0
Grand Forks	5	40	4	0	6	17	15	0	12	0
Griggs	2	50	2	0	1	0	0	-	4	0
Kidder	0	-	0	-	0	-	0	-	4	0
LaMoure	2	50	0	-	0	-	3	0	2	0
Logan	0	-	0	-	0	-	0	-	5	0
McIntosh	0	-	0	-	0	-	0	-	5	0
McKenzie	0	-	4	0	0	-	0	-	0	-
McLean	0	-	1	0	0	-	0	-	0	-
Mountrail	0	-	1	0	0	-	0	-	0	-
Nelson	0	-	0	-	0	-	0	-	4	0
Pierce	0	-	1	0	0	-	0	-	0	-
Ransom	2	50	6	0	6	0	5	0	3	0
Richland	9	33	3	33	10	30	14	0	5	40
Sargent	1	100	6	0	11	18	5	0	4	0
Steele	1	100	2	0	6	50	8	0	11	0
Stutsman	2	100	0	-	0	-	3	0	5	0
Traill	5	0	11	45	8	25	5	0	5	0
Walsh	0	-	0	-	0	-	4	0	8	0
Wells	0	-	0	-	0	-	0	-	3	0
Williams	0	-	5	20	0	-	0	-	1	0
Total	57	37	80	19	82	15	103	0	109	2

[z]Prevalence defined as the number of infected fields divided by the total number of fields surveyed in a county or year and multiplied by 100.

Table 1.4. Prevalence (%) of Goss's leaf blight in North Dakota counties from 2014 to 2018.

County	2014 Fields	Prevalence[z]	2015 Fields	Prevalence	2016 Fields	Prevalence	2017 Fields	Prevalence	2018 Fields	Prevalence
Barnes	5	40	3	0	11	27	10	20	5	80
Burleigh	0	-	0	-	0	-	0	-	4	75
Cass	13	31	25	8	14	57	22	55	9	89
Dickey	5	100	6	0	0	-	3	33	2	100
Eddy	0	-	0	-	0	-	0	-	3	67
Emmons	3	0	0	-	6	50	3	33	3	0
Foster	2	0	0	-	3	100	3	67	2	100
Grand Forks	5	20	4	0	6	0	15	0	12	58
Griggs	2	0	2	0	1	100	0	-	4	25
Kidder	0	-	0	-	0	-	0	-	4	0
LaMoure	2	50	0	-	0	-	3	67	2	100
Logan	0	-	0	-	0	-	0	-	5	60
McIntosh	0	-	0	-	0	-	0	-	5	60
McKenzie	0	-	4	25	0	-	0	-	0	-
McLean	0	-	1	0	0	-	0	-	0	-
Mountrail	0	-	1	0	0	-	0	-	0	-
Nelson	0	-	0	-	0	-	0	-	4	50
Pierce	0	-	1	0	0	-	0	-	0	-
Ransom	2	50	6	0	6	0	5	40	3	100
Richland	9	11	3	0	10	10	14	21	5	60
Sargent	1	100	6	0	11	0	5	0	4	100
Steele	1	0	2	0	6	17	8	75	11	91
Stutsman	2	100	0	-	0	-	3	33	5	60
Traill	5	0	11	18	8	0	5	80	5	80
Walsh	0	-	0	-	0	-	4	0	8	38
Wells	0	-	0	-	0	-	0	-	3	67
Williams	0	-	5	0	0	-	0	-	1	100
Total	57	32	80	6	82	24	103	35	109	66

[z]Prevalence defined as the number of infected fields divided by the total number of fields surveyed in a county or year and multiplied by 100.
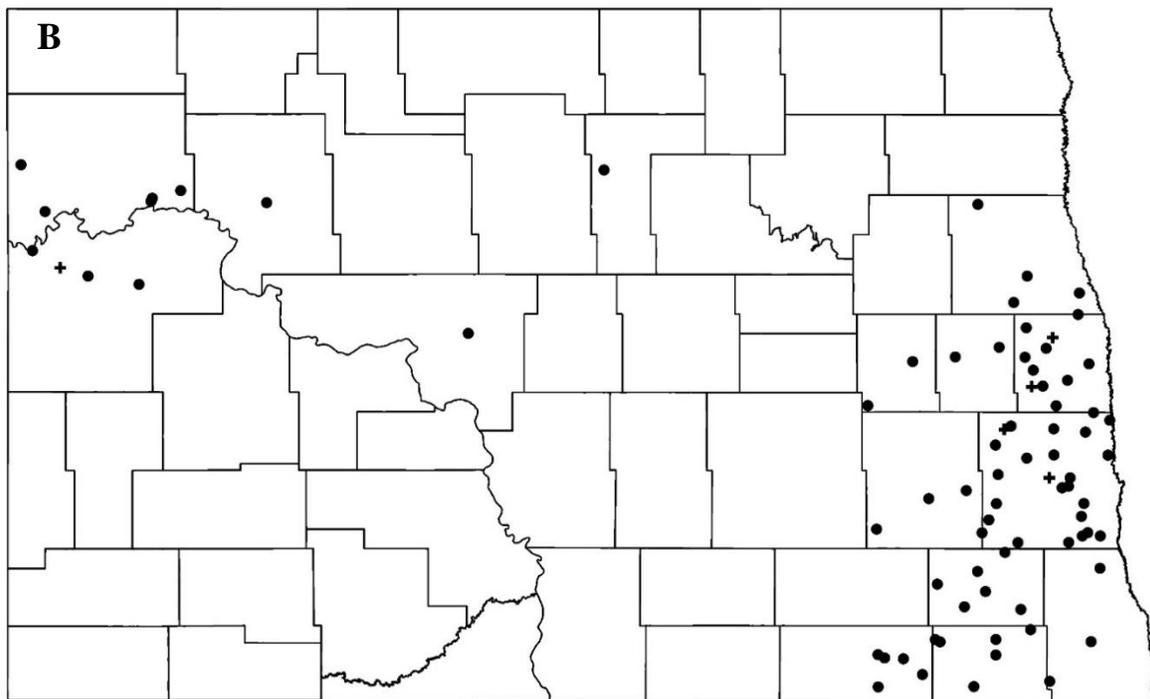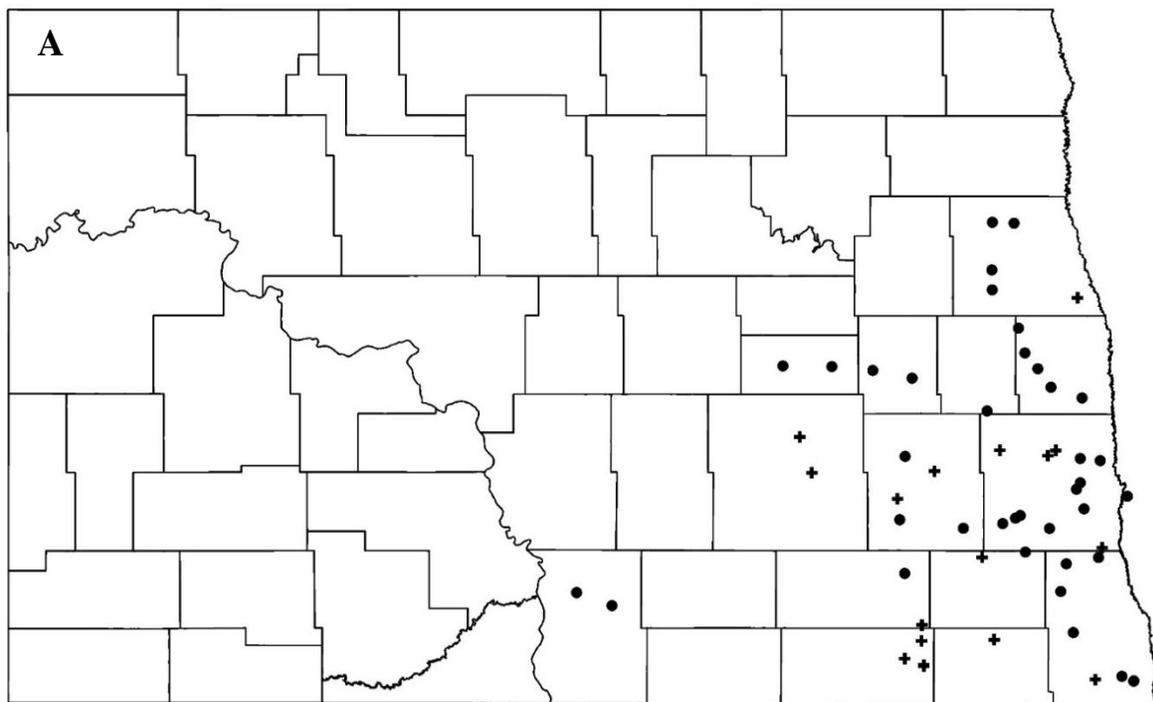
Figure 1.2. Distribution of surveyed fields during the 2014 (A) and 2015 (B) foliar disease surveys of North Dakota corn fields. Locations designated as (●) represent fields where Goss's leaf blight was not identified, while locations designated as (+) represent fields in which Goss's leaf blight was present.
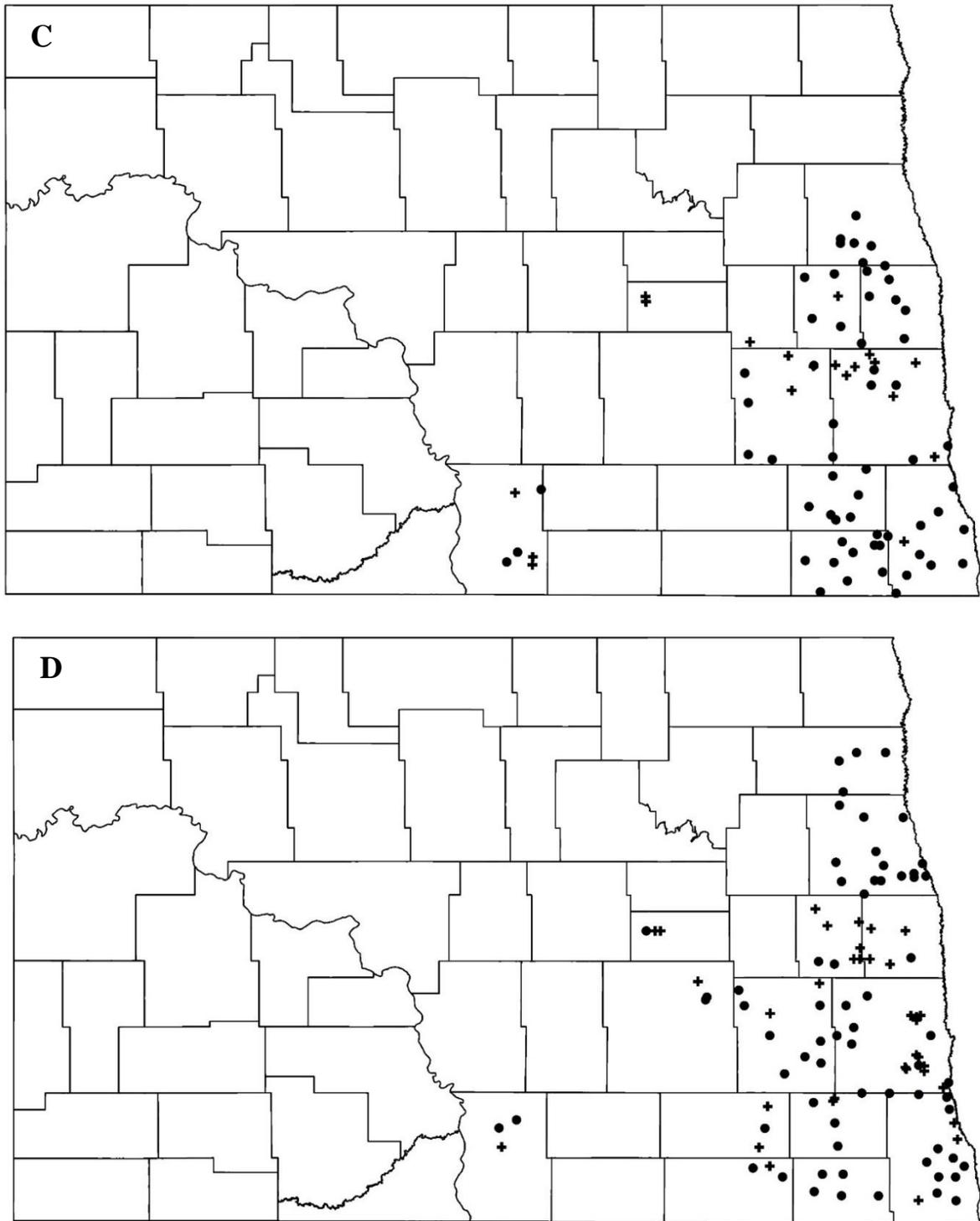
Figure 1.2. Distribution of surveyed fields during the 2016 (C) and 2017 (D) foliar disease surveys of North Dakota corn fields. Locations designated as (●) represent fields where Goss's leaf blight was not identified, while locations designated as (+) represent fields in which Goss's leaf blight was present (continued).
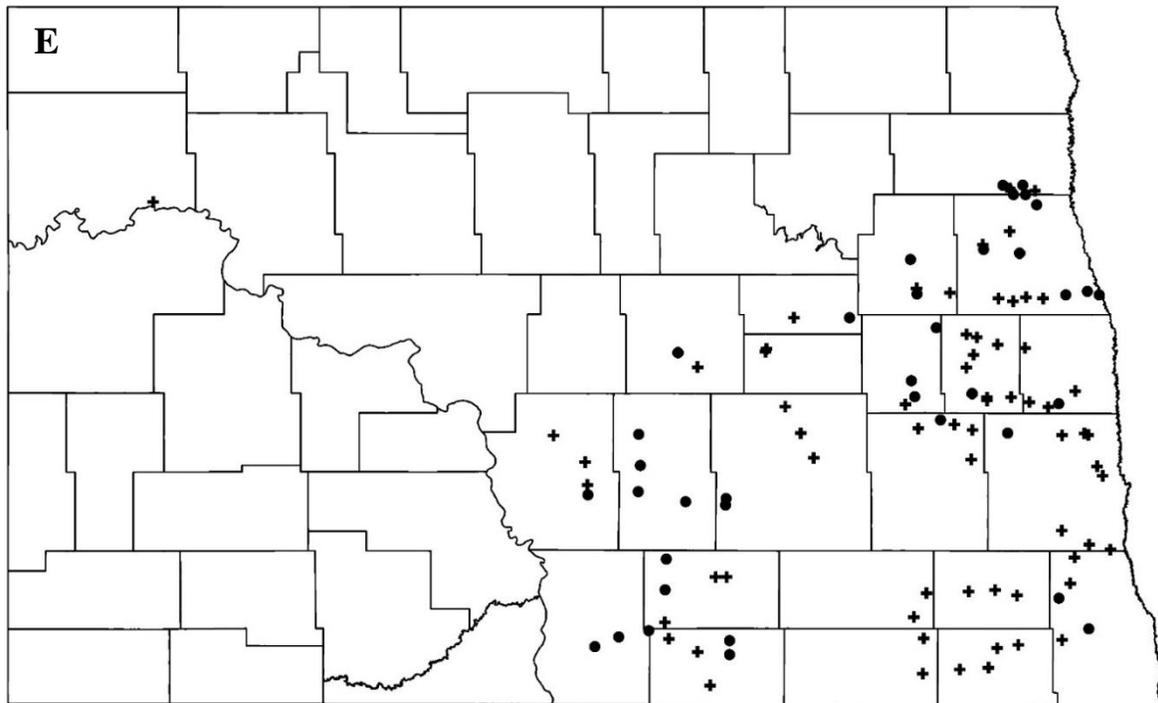
Figure 1.2. Distribution of surveyed fields during the 2018 (E) foliar disease surveys of North Dakota corn fields. Locations designated as (•) represent fields where Goss's leaf blight was not identified, while locations designated as (+) represent fields in which Goss's leaf blight was present (continued).

Chi-square analysis indicated significant differences in disease prevalence among documented diseases. Across all survey years, disease was more likely to be observed in 2014, 2016, 2017, and 2018 than in 2015 (Table 1.5). Total disease prevalence across all five years indicated CR, CS, and GLB were more likely to be observed than NCLB. However, CR was the most likely disease to be found across all survey years (Table 1.6). When data were sorted by each disease across each year, significance in disease prevalence was observed for all four diseases (Table 1.7). When compared among years, CR was more likely to be observed in 2014, 2016, and 2017; CS was most likely to be observed in 2018; NCLB was most likely to be observed in 2014; and GLB was most likely to be found in 2018 and least likely to be found in 2015. Data were also sorted in respect to the top-three corn producing counties in ND; Barnes,

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Cass, and Richland. Results from chi-square indicated that no significant differences were

apparent for CR, CS, NCLB, and GLB prevalence among these counties (data not presented).

Table 1.5. Chi-square analysis comparing disease prevalence differences among years with corresponding statistics.

	2014	2015	2016	2017	2018	*P*-value
Fields with disease (%)	46	24	39	43	43	< 0.0001[y]
Least squares mean	0.38 a	0.16 b	0.33 a	0.37 a	0.42 a	< 0.0001[z]

[y]Level of significance (*P*-value) for chi-square test of homogeneity at the 99% level of confidence.
[z]Level of significance from Tukey-Kramer multiple comparison test. Columns labeled with the same letter are not statistically different ($\alpha = 0.05$).

Table 1.6. Chi-square analysis comparing prevalence differences among common rust, common smut, northern corn leaf blight, and Goss's leaf blight across all fields surveyed during 2014 to 2018 with corresponding statistics.

	CR	CS	NCLB	GLB	*P*-value
Fields with infection (%)	68	45	12	35	< 0.0001[y]
Least squares mean	0.62 a	0.36 b	0.06 c	0.29 b	< 0.0001[z]

[y]Level of significance (*P*-value) for chi-square test of homogeneity at the 99% level of confidence.
[z]Level of significance from Tukey-Kramer multiple comparison test. Columns labeled with the same letter are not statistically different ($\alpha = 0.05$).

Table 1.7. Chi-square analysis comparing prevalence differences for common rust, common smut, northern corn leaf blight and Goss's leaf blight across each year and corresponding statistics.

Disease[x]	2014	2015	2016	2017	2018	*P*-value
Fields with CR (%)	91	40	80	93	41	< 0.0001[y]
Least squares mean	0.82 a	0.34 b	0.73 a	0.87 a	0.38 b	< 0.0001[z]
Fields with CS (%)	25	41	29	44	62	< 0.0001[y]
Least squares mean	0.12 c	0.27 bc	0.14 bc	0.33 b	0.61 a	< 0.0001[z]
Fields with NCLB (%)	37	19	15	0	2	< 0.0001[y]
Least squares mean	0.32 a	0.16 b	0.12 b	-0.04 c	-0.02 c	< 0.0001[z]
Fields with GLB (%)	32	6	24	35	66	< 0.0001[y]
Least squares mean	0.25 b	-0.03 c	0.22 b	0.32 b	0.70 a	< 0.0001[z]

[x]CR = common rust, CS = common smut, NCLB = northern corn leaf blight, GLB = Goss's leaf blight.
[y]Level of significance (*P*-value) for chi-square test of homogeneity at the 99% level of confidence.
[z]Level of significance from Tukey-Kramer multiple comparison test. Columns labeled with the same letter are not statistically different ($\alpha = 0.05$).

The average monthly temperatures (°C) and rainfall (mm) for the corn growing season

months of May through August for all counties and years included in the survey are presented in

Tables 1.8 and 1.9, respectively. As expected, the lowest mean temperatures occurred during the

month of May with the lowest mean temperature recorded in 2015. The highest temperatures

occurred in July with a monthly peak average of 21.8°C occurring in 2017. Average departures

from normal (the 30-year average) ranged from 1.8°C below normal in August of 2017 to 3.2°C

above normal in May of 2018. The highest amount of rain received was in May of 2015 (118.9

mm) and the lowest amount of rain was in July of 2017 (32.7 mm). Average departures from

normal ranged from 46.4 mm below normal rainfall in July of 2017 to 50.9 mm above normal in

May of 2015. Using the weather data and GLB prevalence data, significant negative linear

relationships were found to exist between May 15 to June 15 rainfall and GLB prevalence for

survey years of 2017 and 2018 (Table 1.10). When GLB prevalence data were combined across

all survey years, significant linear relationships (P=0.05) existed with average temperatures

across all three time periods and a significant relationship was observed for rainfall from May 15

to June 15 (Table 1.10). However, the strength of the relationship was generally weak for these

parameters.

Table 1.8. Mean monthly temperatures (°C) and departures from normal (30-year average) for May, June, July, and August of 2014 through 2018. Data is reported for all North Dakota counties included in the 2014 to 2018 corn foliar disease survey.

							May									June				
	2014		2015		2016		2017		2018		2014		2015		2016		2017		2018	
County	M[x]	D[z]	M	D	M	D	M	D	M	D	M	D	M	D	M	D	M	D	M	D
Barnes	12.7	-0.3	11.7	-1.3	14.6	1.6	13.0	0.0	16.3	3.3	18.4	0.3	19.1	0.9	19.2	1.1	18.9	0.7	20.7	2.5
Cass	13.4	-0.2	12.2	-1.4	15.0	1.3	13.3	-0.3	17.1	3.4	19.2	0.5	19.3	0.6	19.8	1.0	19.2	0.4	20.8	2.0
Dickey	13.9	0.6	12.0	-1.2	14.5	1.3	13.3	0.0	17.3	4.0	19.1	0.7	19.9	1.5	20.9	2.5	19.4	1.1	21.3	2.9
Eddy	12.2	-0.6	11.5	-1.2	14.0	1.2	12.4	-0.4	16.0	3.2	17.2	-0.6	18.5	0.7	18.0	0.2	17.9	0.1	20.1	2.3
Emmons	12.8	-0.4	11.9	-1.3	14.0	0.8	13.8	0.6	16.6	3.4	17.1	-1.4	19.1	0.7	19.5	1.1	19.4	1.0	20.3	1.9
Foster	12.4	-0.7	11.3	-1.8	13.6	0.5	12.9	-0.2	16.0	3.0	17.2	-0.8	18.3	0.3	18.2	0.2	18.1	0.1	20.1	2.1
Grand Forks	12.6	-0.5	12.0	-1.2	15.2	2.0	13.0	-0.1	16.0	2.8	18.5	0.3	18.7	0.4	18.7	0.4	18.8	0.6	20.1	1.9
Griggs	.[y]	.	11.4	-0.9	14.2	1.9	12.0	-0.3	15.4	3.1	.	.	18.6	1.2	19.4	2.0	18.0	0.6	19.6	2.2
Kidder	12.4	-0.3	11.5	-1.1	13.6	1.0	12.1	-0.5	15.4	2.8	16.9	-0.8	18.5	0.8	18.6	0.9	17.6	-0.1	19.5	1.8
LaMoure	13.2	-0.2	11.8	-1.6	14.2	0.9	13.0	-0.4	16.6	3.2	18.6	0.1	19.7	1.2	19.6	1.2	19.0	0.5	20.9	2.4
McIntosh	12.1	0.1	11.1	-1.0	13.2	1.1	12.4	0.3	15.6	3.5	16.3	-0.7	18.4	1.3	18.4	1.4	18.3	1.3	19.2	2.1
McKenzie	12.4	0.6	11.6	-0.3	13.5	1.7	13.3	1.5	15.5	3.7	16.4	-0.7	19.0	1.9	19.3	2.2	18.2	1.1	19.0	1.9
McLean	12.0	-0.1	11.5	-0.6	13.8	1.7	12.8	0.8	15.8	3.8	16.4	-0.8	18.1	1.0	18.2	1.1	18.0	0.8	19.1	1.9
Mountrail	11.8	0.0	10.9	-0.9	13.1	1.3	12.4	0.7	15.2	3.4	15.9	-1.0	17.9	1.0	17.7	0.8	17.1	0.2	18.3	1.4
Nelson	12.8	0.8	10.8	-1.2	13.5	1.1	11.8	-0.6	15.2	2.8	18.1	0.8	18.4	0.8	17.5	0.0	17.4	-0.2	19.0	1.5
Pierce	12.0	-0.1	11.3	-0.8	14.4	2.4	12.9	0.8	15.3	3.2	17.1	-0.2	17.6	0.4	18.5	1.2	17.7	0.4	19.2	1.9
Ransom	13.3	-0.5	12.6	-1.2	15.2	1.4	13.5	-0.3	17.3	3.5	18.8	-0.4	19.9	0.7	20.9	1.7	19.6	0.4	21.0	1.9
Richland	13.6	-0.8	12.5	-2.0	15.1	0.7	13.7	-0.8	17.6	3.2	19.4	-0.1	19.7	0.3	19.9	0.5	19.3	-0.1	20.9	1.5
Sargent	.	.	12.0	-1.6	15.0	1.4	12.5	-1.1	16.8	3.1	.	.	19.7	0.8	20.1	1.3	18.3	-0.5	20.6	1.8
Steele	.	.	11.6	-0.9	14.4	2.0	12.3	-0.1	15.9	3.4	.	.	18.7	1.3	18.5	1.1	18.1	0.7	19.9	2.5
Stutsman	12.6	-0.2	11.9	-1.0	14.2	1.3	12.5	-0.3	15.9	3.1	17.8	-0.4	19.3	1.2	18.7	0.6	18.2	0.0	19.6	1.5
Traill	12.8	-0.4	11.9	-1.2	14.7	1.5	12.9	-0.2	16.4	3.3	18.6	0.2	19.0	0.6	19.0	0.5	18.8	0.4	20.6	2.1
Walsh	12.5	-0.4	11.7	-1.3	14.7	1.7	12.7	-0.2	15.8	2.9	18.3	0.2	18.4	0.3	18.4	0.3	18.4	0.3	19.8	1.7
Wells	12.3	-0.6	11.4	-1.5	13.7	0.8	12.6	-0.2	15.7	2.8	17.2	-0.6	18.3	0.5	18.2	0.4	17.8	0.0	19.5	1.8
Williams	12.4	-0.7	11.5	-1.6	13.8	0.7	13.2	0.7	15.4	2.9	16.6	-1.4	18.8	0.8	19.1	1.1	17.7	0.3	18.7	1.3
Overall mean	12.6	-0.2	11.7	-1.2	14.2	1.3	12.8	0.0	16.1	3.2	17.7	-0.3	18.8	0.8	19.0	1.0	18.4	0.4	19.9	2.0

[x]M = mean monthly temperature (°C).
[y]Missing data for counties without weather stations are represented by (.).
[z]D = departure from normal (30-year average) (°C).

Table 1.8. Mean monthly temperatures (°C) and departures from normal (30-year average) for May, June, July, and August of 2014 through 2018. Data is reported for all North Dakota counties included in the 2014 to 2018 corn foliar disease survey (continued).

| | July | | | | | | | | | | August | | | | | | | | | |
| | 2014 | | 2015 | | 2016 | | 2017 | | 2018 | | 2014 | | 2015 | | 2016 | | 2017 | | 2018 | |
County	M[x]	D[z]	M	D	M	D	M	D	M	D	M	D	M	D	M	D	M	D	M	D
Barnes	19.6	-1.3	21.1	0.3	20.6	-0.2	21.4	0.5	20.6	-0.3	19.5	-0.4	19.3	-0.6	19.6	-0.3	18.3	-1.6	18.9	-1.0
Cass	20.2	-1.3	21.7	0.3	21.3	-0.2	21.5	0.1	20.9	-0.6	20.3	-0.2	19.8	-0.7	20.3	-0.1	18.6	-1.9	19.6	-0.9
Dickey	20.1	-1.3	21.8	0.3	22.7	1.2	22.1	0.6	21.1	-0.4	20.1	-0.3	20.0	-0.3	21.7	1.4	18.4	-1.9	19.7	-0.6
Eddy	19.4	-1.3	20.7	0.0	20.3	-0.4	21.8	1.1	20.8	0.1	19.6	-0.3	20.2	0.3	19.6	-0.3	19.1	-0.8	19.7	-0.1
Emmons	20.1	-1.9	21.5	-0.5	22.0	0.0	23.4	1.4	21.2	-0.7	19.9	-1.0	20.7	-0.3	20.8	-0.2	18.7	-2.2	20.6	-0.3
Foster	18.6	-2.4	20.5	-0.4	20.5	-0.4	21.5	0.5	20.3	-0.7	18.3	-1.6	19.1	-0.9	19.5	-0.4	18.0	-1.9	19.2	-0.7
Grand Forks	19.7	-1.2	21.3	0.4	20.7	-0.2	21.3	0.4	20.7	-0.3	19.6	-0.3	19.8	0.0	20.3	0.4	18.3	-1.6	19.5	-0.4
Griggs	.[y]	.	20.9	1.0	21.3	1.4	20.9	1.0	20.3	0.4	.	.	19.6	0.6	20.4	1.3	18.1	-0.9	18.8	-0.2
Kidder	19.2	-1.9	21.4	0.3	20.8	-0.3	22.2	1.1	20.3	-0.8	19.4	-1.0	20.6	0.3	19.4	-0.9	18.0	-2.3	19.5	-0.8
LaMoure	19.7	-1.8	21.8	0.3	21.0	-0.5	21.8	0.3	20.6	-0.9	19.7	-0.9	20.1	-0.5	19.7	-0.9	18.0	-2.6	19.0	-1.6
McIntosh	19.1	-1.3	21.0	0.6	20.4	0.0	22.2	1.8	20.2	-0.2	19.1	-0.3	20.1	0.7	19.6	0.2	17.7	-1.7	19.5	0.1
McKenzie	20.5	-0.3	22.0	1.2	21.8	1.0	24.2	3.4	20.8	0.1	20.3	0.4	21.3	1.4	21.3	1.4	19.6	-0.3	20.5	0.6
McLean	19.2	-1.2	20.7	0.4	20.7	0.3	22.6	2.2	20.1	-0.3	19.5	-0.3	20.5	0.7	20.4	0.7	18.5	-1.2	20.1	0.3
Mountrail	18.6	-1.6	20.2	0.0	19.6	-0.6	21.7	1.5	19.0	-1.2	19.1	-0.6	19.7	0.0	19.3	-0.4	17.8	-1.9	19.1	-0.6
Nelson	20.0	0.1	20.5	0.3	19.7	-0.6	20.5	0.2	19.8	-0.4	19.0	-0.2	19.2	-0.1	19.0	-0.3	17.5	-1.9	18.7	-0.6
Pierce	19.3	-0.6	20.7	0.8	20.1	0.2	21.4	1.5	19.8	-0.1	19.2	0.0	19.7	0.5	19.5	0.3	18.0	-1.2	19.4	0.1
Ransom	19.8	-2.3	22.2	0.1	22.5	0.5	22.1	0.1	21.3	-0.8	20.1	-1.0	20.6	-0.4	20.8	-0.3	18.9	-2.2	19.8	-1.2
Richland	20.3	-1.8	21.7	-0.3	21.6	-0.5	21.3	-0.7	21.1	-0.9	20.4	-0.7	20.1	-1.1	20.7	-0.5	18.4	-2.8	19.8	-1.3
Sargent	.	.	21.6	-0.2	21.8	0.0	21.3	-0.6	20.9	-0.9	.	.	19.7	-1.1	20.4	-0.3	17.8	-3.0	19.5	-1.3
Steele	.	.	21.0	1.2	20.5	0.6	20.7	0.9	20.2	0.4	.	.	19.6	0.2	19.9	0.4	17.9	-1.5	18.9	-0.6
Stutsman	19.5	-1.8	21.6	0.3	20.6	-0.7	21.5	0.2	20.5	-0.8	19.4	-0.7	20.0	-0.1	19.3	-0.8	18.0	-2.1	18.7	-1.4
Traill	19.6	-1.4	20.9	-0.1	20.7	-0.3	21.0	-0.1	20.6	-0.4	19.8	-0.3	19.1	-0.9	19.7	-0.3	18.2	-1.9	19.3	-0.7
Walsh	19.3	-1.3	21.3	0.7	20.5	-0.1	20.6	0.0	20.7	0.0	19.7	0.0	19.6	-0.1	19.7	0.0	18.0	-1.7	19.6	-0.1
Wells	19.1	-1.7	20.8	0.0	20.3	-0.5	22.1	1.3	20.0	-0.8	18.8	-1.1	19.6	-0.3	19.3	-0.6	18.2	-1.7	19.4	-0.5
Williams	20.1	-1.5	21.7	0.2	21.0	-0.5	22.8	2.0	19.9	-1.0	20.1	-0.9	20.9	-0.2	20.5	-0.6	18.9	-1.5	19.6	-0.8
Overall mean	19.6	-1.4	21.2	0.3	20.9	0.0	21.8	0.8	20.5	-0.5	19.6	-0.5	20.0	-0.1	20.0	0.0	18.3	-1.8	19.5	-0.6

[x]M = mean monthly temperature (°C).
[y]Missing data for counties without weather stations are represented by (.).
[z]D = departure from normal (30-year average) (°C).

Table 1.9. Mean monthly rainfall (mm) and departures from normal (30-year average) for May, June, July, and August of 2014 through 2018. Data is reported for all North Dakota counties included in the 2014 to 2018 corn foliar disease survey.

| | May | | | | | | | | | | June | | | | | | | | | |
| | 2014 | | 2015 | | 2016 | | 2017 | | 2018 | | 2014 | | 2015 | | 2016 | | 2017 | | 2018 | |
County	M[y]	D[z]	M	D	M	D	M	D	M	D	M	D	M	D	M	D	M	D	M	D
Barnes	51.7	-23.4	149.0	73.9	79.1	4.0	25.3	-49.8	53.8	-21.3	109.9	22.7	100.1	12.9	80.1	-7.1	76.5	-10.7	90.3	3.1
Cass	47.2	-27.1	146.0	71.8	69.1	-5.1	20.3	-53.9	39.4	-34.9	124.9	31.8	91.4	-1.7	49.7	-43.4	59.0	-34.2	85.8	-7.4
Dickey	46.8	-28.4	152.3	77.1	51.4	-23.8	37.1	-38.1	21.7	-53.5	154.4	58.6	81.0	-14.8	65.5	-30.3	54.3	-41.5	134.6	38.8
Eddy	55.6	-9.9	127.6	62.1	72.2	6.7	30.5	-35.1	36.6	-29.0	125.2	33.3	87.9	-4.0	115.1	23.2	91.7	-0.2	95.3	3.4
Emmons	21.2	-44.1	134.4	69.1	90.7	25.5	14.7	-50.5	77.0	11.7	132.9	56.9	142.6	66.6	20.3	-55.6	68.4	-7.6	89.2	13.3
Foster	40.2	-29.9	114.9	44.8	29.1	-41.0	23.9	-46.2	32.4	-37.7	84.4	-11.3	53.2	-42.5	44.8	-51.0	92.0	-3.8	117.7	21.9
Grand Forks	65.7	-3.5	114.0	44.7	96.6	27.4	25.5	-43.7	57.4	-11.8	149.5	61.3	94.8	6.6	108.8	20.7	151.1	63.0	112.8	24.7
Griggs	.	.	132.9	58.8	100.9	26.7	32.5	-41.7	62.0	-12.1	.	.	76.7	-10.4	92.0	4.9	123.0	35.9	59.2	-27.9
Kidder	23.9	-38.2	128.8	66.7	30.6	-31.6	14.7	-47.4	78.2	16.0	117.9	33.1	82.6	-2.2	49.6	-35.2	82.3	-2.6	119.0	34.2
LaMoure	68.2	-8.2	162.8	86.5	79.9	3.6	34.9	-41.4	71.6	-4.8	112.0	26.0	102.5	16.6	105.3	19.4	32.4	-53.6	80.4	-5.5
McIntosh	48.5	-16.8	166.0	100.7	77.8	12.5	10.4	-54.9	79.0	13.8	86.3	10.3	83.4	7.4	50.8	-25.1	28.7	-47.2	82.6	6.6
McKenzie	74.7	18.8	36.3	-19.6	50.6	-5.3	21.7	-34.2	30.7	-25.1	56.9	-19.3	103.1	26.9	22.0	-54.2	59.9	-16.3	70.7	-5.5
McLean	72.2	7.2	50.6	-14.4	66.2	1.1	17.0	-48.0	28.9	-36.1	126.9	34.7	124.3	32.1	58.8	-33.4	23.0	-69.2	158.2	66.0
Mountrail	47.0	-17.0	38.5	-25.5	59.7	-4.3	25.5	-38.5	40.1	-23.9	114.3	23.5	82.6	-8.2	72.7	-18.1	21.3	-69.5	191.3	100.5
Nelson	60.9	-2.3	126.3	63.1	89.9	26.6	35.7	-27.5	43.2	-20.1	140.4	44.9	101.6	5.6	110.0	14.0	69.1	-26.9	111.7	15.7
Pierce	62.5	-9.9	66.8	-5.6	39.4	-33.0	13.0	-59.4	39.4	-33.0	114.3	25.4	87.2	-1.7	104.4	15.5	73.5	-15.4	131.4	42.5
Ransom	49.8	-25.1	154.0	79.1	66.9	-8.1	31.5	-43.4	24.4	-50.5	112.0	31.8	90.7	10.5	39.1	-41.1	51.3	-28.9	90.5	10.2
Richland	71.1	-4.5	177.6	102.0	39.6	-36.0	33.5	-42.1	15.4	-60.2	129.8	36.5	81.6	-11.7	29.4	-63.9	75.7	-17.6	120.9	27.6
Sargent	.	.	162.4	93.0	66.0	-3.4	20.4	-48.9	22.0	-47.4	.	.	40.3	-62.4	45.8	-56.8	83.4	-19.2	119.5	16.9
Steele	.	.	105.9	38.1	69.6	1.8	34.2	-33.6	74.1	6.3	.	.	108.0	12.8	93.0	-2.2	80.8	-14.4	54.6	-40.6
Stutsman	76.7	8.9	143.3	75.5	50.6	-17.2	21.8	-46.0	69.4	1.5	113.2	25.3	137.0	49.1	59.2	-28.7	58.7	-29.2	144.6	56.7
Traill	41.3	-27.2	100.9	32.4	99.5	31.1	23.7	-44.7	41.3	-27.2	160.1	66.9	106.5	13.3	36.9	-56.4	81.2	-12.0	85.4	-7.8
Walsh	74.6	4.6	141.7	71.7	106.7	36.8	32.0	-38.0	73.0	3.0	160.1	69.2	99.7	8.8	176.8	85.9	91.3	0.4	82.3	-8.7
Wells	35.5	-24.2	104.9	45.2	32.3	-27.4	12.7	-47.0	34.0	-25.7	76.5	-10.2	66.7	-19.9	43.2	-43.5	70.7	-16.0	128.3	41.7
Williams	43.8	-10.3	35.4	-18.7	62.6	8.5	28.2	-25.7	47.7	-6.2	54.9	-19.2	55.9	-18.1	54.7	-19.3	33.2	-41.2	106.7	32.3
Overall mean	53.6	-14.1	118.9	50.9	67.1	-1.0	24.8	-43.2	47.7	-20.3	116.2	28.7	91.3	2.9	69.1	-19.3	69.3	-19.1	106.5	18.1

[x]M = mean monthly temperature (°C).
[y]Missing data for counties without weather stations are represented by (.).
[z]D = departure from normal (30-year average) (°C).

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Table 1.9. Mean monthly rainfall (mm) and departures from normal (30-year average) for May, June, July, and August of 2014 through 2018. Data is reported for all North Dakota counties included in the 2014 to 2018 corn foliar disease survey (continued).

	July										August									
	2014		2015		2016		2017		2018		2014		2015		2016		2017		2018	
County	M[y]	D[z]	M	D	M	D	M	D	M	D	M	D	M	D	M	D	M	D	M	D
Barnes	16.2	-65.3	66.6	-14.8	112.1	30.7	42.6	-38.8	83.4	1.9	116.2	50.9	45.4	-19.8	70.4	5.1	71.8	6.5	89.3	24.0
Cass	35.8	-43.9	70.4	-9.2	101.2	21.5	37.8	-41.8	74.0	-5.7	70.5	5.2	39.5	-25.9	47.9	-17.4	45.7	-19.7	86.2	20.9
Dickey	51.1	-30.7	19.6	-62.2	140.2	58.4	22.3	-59.5	194.7	112.9	125.5	65.5	33.1	-26.9	128.5	68.5	98.0	38.0	36.0	-24.0
Eddy	49.8	-36.8	108.3	21.6	119.7	33.1	9.1	-77.5	91.4	4.8	73.4	0.8	33.0	-39.6	91.0	18.3	56.9	-15.7	2.3	-70.4
Emmons	28.0	-41.3	53.4	-16.0	129.4	60.0	50.1	-19.3	106.2	36.9	156.5	104.6	53.1	1.3	71.4	19.6	99.6	47.8	30.8	-21.1
Foster	65.4	-20.7	112.6	26.5	114.9	28.8	28.7	-57.4	67.4	-18.7	45.3	-13.4	42.9	-15.8	93.0	34.4	87.7	29.0	6.1	-52.6
Grand Forks	47.5	-35.3	118.1	35.3	135.1	52.3	18.0	-64.8	67.2	-15.6	157.1	88.9	57.2	-11.0	74.1	5.9	44.2	-24.0	24.4	-43.8
Griggs	.	.	97.3	6.4	150.0	59.0	53.9	-37.1	74.0	-17.0	.	.	79.3	7.4	75.2	3.3	91.7	19.9	89.7	17.9
Kidder	39.7	-40.9	28.0	-52.6	139.6	59.0	15.3	-65.3	138.4	57.8	101.3	46.6	27.2	-27.6	72.8	18.0	101.2	46.4	31.9	-22.9
LaMoure	25.8	-51.8	53.0	-24.6	82.0	4.4	24.8	-52.8	174.0	96.4	82.5	17.8	31.8	-32.9	83.0	18.3	89.2	24.6	39.3	-25.4
McIntosh	29.6	-39.7	30.5	-38.9	177.8	108.5	54.9	-14.5	98.1	28.8	105.2	53.4	36.6	-15.2	61.0	9.2	74.4	22.6	41.4	-10.4
McKenzie	18.1	-47.7	39.2	-26.6	46.3	-19.5	22.0	-43.8	73.5	7.7	64.9	28.8	37.1	1.0	19.6	-16.5	43.2	7.1	34.0	-2.0
McLean	40.6	-30.7	70.4	-0.9	75.0	3.6	20.5	-50.9	57.1	-14.2	127.9	77.0	23.5	-27.5	22.3	-28.6	74.6	23.7	25.9	-25.1
Mountrail	38.7	-35.8	94.2	19.6	95.7	21.1	32.4	-42.2	62.3	-12.3	111.8	61.3	38.8	-11.7	15.1	-35.3	46.1	-4.3	32.3	-18.2
Nelson	112.5	30.7	124.4	39.3	124.7	39.6	24.8	-60.3	72.6	-12.5	90.4	20.6	32.4	-39.7	102.2	30.0	70.7	-1.5	12.3	-59.8
Pierce	40.6	-45.0	63.8	-21.8	104.5	18.9	52.6	-33.0	33.0	-52.6	69.3	14.7	18.6	-36.0	29.5	-25.1	60.2	5.6	36.9	-17.8
Ransom	17.3	-62.5	35.1	-44.7	81.3	1.6	17.3	-62.5	95.6	15.8	127.5	73.9	43.5	-10.1	128.4	74.8	97.1	43.5	20.8	-32.8
Richland	24.9	-61.3	45.4	-40.9	119.0	32.7	49.6	-36.7	107.5	21.2	84.8	27.5	27.1	-30.2	92.4	35.0	113.8	56.5	58.2	0.8
Sargent	.	.	25.7	-58.2	145.7	61.9	18.8	-65.0	167.9	84.1	.	.	35.8	-18.6	51.4	-3.0	171.1	116.8	48.9	-5.4
Steele	.	.	106.5	27.3	112.0	32.8	89.1	9.8	62.0	-17.2	.	.	33.3	-35.3	54.4	-14.2	37.2	-31.4	96.6	28.0
Stutsman	23.4	-60.2	88.2	4.6	133.2	49.6	29.7	-53.8	101.6	18.1	62.0	7.9	26.4	-27.7	95.8	41.7	100.2	46.1	109.1	55.0
Traill	46.2	-36.7	80.2	-2.8	110.7	27.8	52.6	-30.3	64.6	-18.4	83.7	19.8	33.4	-30.5	123.1	59.3	12.3	-51.6	73.9	10.1
Walsh	46.8	-30.9	140.1	62.4	160.5	82.8	19.9	-57.8	70.6	-7.1	72.3	1.8	152.4	82.0	69.8	-0.7	49.2	-21.3	16.5	-54.0
Wells	32.3	-35.1	51.1	-16.2	95.3	28.0	11.4	-55.9	45.2	-22.1	47.0	-16.3	38.2	-25.1	55.8	-7.4	87.5	24.2	16.0	-47.2
Williams	25.4	-40.6	66.3	0.3	54.2	-11.8	19.7	-48.6	59.3	-8.9	71.9	30.7	30.1	-11.0	10.3	-30.9	64.5	24.0	19.6	-20.9
Overall mean	38.9	-39.2	71.5	-7.5	114.4	35.4	32.7	-46.4	89.7	10.6	93.0	34.9	42.0	-17.1	69.5	10.5	75.5	16.5	43.1	-15.9

[x]M = mean monthly temperature (°C).
[y]Missing data for counties without weather stations are represented by (.).
[z]D = departure from normal (30-year average) (°C).

Table 1.10. Relationships between average temperature (°C) and total rainfall (mm) with Goss's leaf blight prevalence during May 15 to June 15, June 15 to July 15, and July 15 to August 15 in 2014 to 2018. Values represent Pearson correlation coefficients. Values followed by an asterisk (*) represent a significant linear relationship between variables at $P = 0.05$.

Survey year	Average Temperature (°C)			Total Rainfall (mm)		
	May 15 to June 15	June 15 to July 15	July 15 to August 15	May 15 to June 15	June 15 to July 15	July 15 to August 15
2014	0.41	0.24	-0.03	0.03	-0.16	-0.10
2015	-0.07	0.34	0.27	-0.09	0.02	-0.12
2016	-0.38	-0.15	-0.08	-0.01	0.29	-0.15
2017	-0.43	0.02	-0.01	-0.79*	0.25	-0.15
2018	0.39	0.07	-0.11	-0.53*	0.17	0.22
2014 to 2018[z]	0.56*	0.32*	-0.42*	-0.24*	0.23	-0.11

[z]Prevalence and weather data combined for all five years, 2014 to 2018.

Discussion

Across five survey years, CR, CS, NCLB, and GLB were found to be the most common diseases in ND corn fields. Other diseases such as holcus leaf spot, Fusarium ear mold, and Fusarium stalk rot were documented infrequently and appear to be minor problems in ND at this time. Significantly more disease was documented in 2014, 2016, and 2017 than in 2015 or 2018. Lower levels of disease in 2015 were likely due to dry conditions. Across all surveyed fields, CR was the most prevalent corn disease in ND.

The survey results have identified the most important corn disease in ND; GLB. Predominately, the disease was found to be aggregated within fields. Moderate to very high levels of disease were observed in several fields across the five years and substantial yield loss occurred. One of the most severely affected fields reported yield losses in excess of 3,100 kg/ha (G. Endres, personal communication). To note, as evidenced by the distribution of GLB across years (Figure 1.2), it appears that pockets of GLB are re-occurring in a few ND counties (Cass, Foster, Steele, and Traill) each year. *Clavibacter nebraskensis* may be overwintering on infested residue in these areas and is likely spread via wind to nearby fields the following year. The identification of CR and NCLB is also important for developing management strategies. Both of

these diseases can be effectively managed with hybrid resistance, and fungicides are currently

not needed to protect yield. Although Mueller et al. (2016), indicated NCLB as one of the most

important diseases in the U.S., high severity levels are not regularly observed in ND at this time.

In fields where NCLB was identified, only a few plants exhibited symptoms (low incidence) and

it was often identified late in the growing season at a time when no yield loss would be observed.

The prevalence of GLB was highest in 2018 when compared to the other four survey years. The

high level of disease is concerning as susceptible hybrids can suffer severe yield losses when

infection occurs early in the growing season. Also, it appears GLB is becoming readily apparent

in ND as confirmation of GLB occurred for the first time in the counties of Eddy, Logan,

McIntosh, Nelson, and Wells. Throughout this survey effort, GLB was identified in 23 of the 27

surveyed counties and will impact disease management decisions in the future. For example,

creating awareness of the disease has already prompted several growers to use GLB resistant

hybrids, especially in areas that have a short rotation away from corn.

Results from the correlation analyses indicated significant negative linear relationships

were present between GLB prevalence and total rainfall in May 15 to June 15. Another

significant negative relationship was apparent between GLB prevalence and July 15 to August

15 temperatures. In other words, GLB prevalence decreased as rainfall increased in May to June

or GLB prevalence decreased as temperatures increased in July to August. Conducive weather

conditions for *C. nebraskensis* infection is under high humidity and warm temperatures. It is

possible that these weather factors limited GLB, however we believe that the weather data is

likely not representing other factors that promote the development of GLB. In a survey effort in

Nebraska and Iowa, factors such as planting population and hybrid resistance rating were found

to be greater influences of the development of GLB (Langemeier et al. 2017). The risk for the

development of GLB increases with the planting of more susceptible hybrids. High plant populations aid in the spread of disease as well as promote conducive conditions for disease development. The physical contact of leaves enables bacteria to easily move within and between rows and increases humidity within the canopy. In a study looking at the dissemination of *C. nebraskensis* from an inoculum point source, it was reported that new symptoms were observed on plants that were in close proximity to symptomatic plants (Eggenberger et al. 2016). High levels of relative humidity in a greenhouse study were associated with increased population densities of epiphytic *C. nebraskensis* (Mallowa et al. 2016). Other contributing factors to bacterial diseases are hail and strong winds that provide wounds the bacterium can enter. This is very difficult to assess with NDAWN data and is best observed on a field-to-field basis. Given our results, we believe that agronomic factors are likely having a bigger influence than weather on explaining the prevalence increases of GLB.

Moving forward, survey efforts will continue to be beneficial in monitoring the prevalence and distribution of corn disease in ND. Common rust, common smut, northern corn leaf blight, and Goss's leaf blight will likely continue to be found in ND. There is risk for GLB to increase in prevalence and severity if corn is grown in short rotation and/or susceptible hybrids are grown, especially in areas with an abundance of over-wintering inoculum. Finally, first reports of bacterial leaf streak and tar spot in the U.S. have recently been made and it will be necessary to monitor ND corn fields for these diseases as well (Damicone et al. 2018; Korus et al. 2017; Ruhl et al. 2016).

Ways to expand on corn disease surveys include measuring severity (percentage of infected plant area) and incidence (percentage of infected plants in a field) of disease(s) within fields. Goss's wilt and leaf blight is often aggregated within fields, so systematic sampling would

be most appropriate for assessing disease severity and incidence (Byamukama et al. 2011; Eggenberger et al. 2016). Obtaining numeric values of severity and incidence can help estimate yield loss. Mueller et al. (2016) reported that yield loss estimates were determined using data from research trials, disease surveys, information gathered from Extension personnel and university diagnostic laboratories, and informed "guesses". Utilizing distributed surveys similar to Langemeier et al. (2017) could help in gathering agronomic data, such as cropping history, hybrid information, seeding rates, and field disease history from growers. Survey efforts will continue to be a focal point of gathering information on corn diseases in ND. The information provided by surveys helps determine the impact (yield loss) of disease(s) on corn in ND and will direct research efforts focused on alleviating economic losses.

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CHAPTER 2. AGGRESSIVENESS AND GENETIC DIVERSITY OF NORTH DAKOTA

***CLAVIBACTER NEBRASKENSIS* ISOLATES**

<u>Introduction</u>

Corn production in North Dakota (ND) increased from 890,000 hectares planted for grain

in 2011 to 1.4 million hectares in 2017 (NASS 2018). The biggest disease constraint for corn

growers in ND is Goss's wilt and leaf blight caused by the gram positive bacterium *Clavibacter*

nebraskensis (Vidaver and Mandel 1974) Li et al. 2018. The disease was first documented in ND

in 2011, and now has been documented in all major corn producing regions in the state.

Clavibacter nebraskensis survives on corn residue, alternative grass hosts, and marginally on

seed (Biddle et al. 1990; Ikley et al. 2015; Langemeier et al. 2014; Schuster 1975). Infection

primarily occurs through wounds and natural openings (Eggenberger et al. 2016; Mallowa et al.

2016) disrupting photosynthetic activity in leaves and vascular tissue in the stalk. Observations

of lesion progression from the leaf apex downward on seemingly non-wounded leaves have been

reported (Mallowa et al. 2016). The bacterium is disseminated within and between fields via

wind and water droplets, with aerosols providing a possible explanation for long distance

dispersal (Graham and Harrison 1975; Venette and Kennedy 1975). Epiphytic populations of *C.*

nebraskensis have also been identified and can increase in density throughout a season but have

also been found to go undetected over periods of time (Eggenberger et al. 2016; Smidt and

Vidaver 1986).

Several groups have investigated genetic variability among and between populations of

other *Clavibacter* species. Multilocus sequence analysis (MLSA) and repetitive sequence-based

(rep-PCR) genomic fingerprinting documented introductions and the spread of *C. michiganensis*

subsp. *michiganensis* in Turkey and Argentina, respectively (Sen et al. 2018; Wassermann et al.

2017). In Turkey, 108 *C. michiganensis* subsp. *michiganensis* strains collected over a 16-year span were found to be genetically similar (Sen et al. 2018). The genetic uniformity within the population supported the idea of an initial introduction and rapid dissemination of *C. michiganensis* subsp. *michiganensis* in Turkey. Multilocus sequence typing (MSLT) analysis of *C. michiganensis* subsp. *michiganensis* housekeeping genes revealed that the majority of the strains fell into a single group that was widespread throughout Turkey. Only strains isolated after 2004 fell into other groups, indicating few other isolated introductions of *C. michiganensis* subsp. *michiganensis* into Turkey (Sen et al. 2018). A genetic analysis of *C. michiganensis* subsp. *michiganensis* in Argentina indicated new introductions occur every year (Wasserman et al. 2017). Twelve *C. michiganensis* subsp. *michiganensis* strains collected from five greenhouse locations over a span of 14 years were genetically diverse. The 12 strains fell into three distinct groups, although the grouping was not associated spatially or temporally. Strains from multiple groups were present in each greenhouse, indicating multiple sources of inoculum or multiple introductions of *C. michiganensis* subsp. *michiganensis*.

Limited studies on assessing the genetic and phenotypic variation in *C. nebraskensis* populations have been conducted. Bacteriophage and bacteriocin typing were used to group 85 *C. nebraskensis* strains collected between 1969 and 1979 (Vidaver et al. 1981). Although both methods classified the strains into eight groups, no correlation was found among groupings with regards to year of isolation or geographic origin of the strain. In 2011, the first study using molecular techniques to examine genetic diversity among *C. nebraskensis* isolates was published (Agarkova et al. 2011). Amplified fragment length polymorphism (AFLP) analysis and repetitive DNA sequence-based BOX-PCR were used to analyze 131 isolates collected between 1969 and 2009. The isolates clustered into two groups; 118 isolates in group A and 13 isolates in group B.

A composite analysis of data from both the AFLP analysis and BOX-PCR showed that the genome of Group A had been stable for a long period of time. No correlations were present between origin, morphology, or physiology (defined by results of Gram stain and KOH test, *C. nebraskensis* is gram positive and negative for the KOH test) for the isolates in Group A. All 13 isolates in Group B were collected after 1999, and represented recent genetic changes between 1999 and 2009 (Agarkova et al. 2011). A greenhouse study comparing *C. nebraskensis* isolates isolated from symptomatic and asymptomatic corn leaves found differences in aggressiveness, in terms of proportion of leaf area infected, among isolates (Ahmad et al. 2015). However, no relationship existed between aggressiveness and the origin of the isolate (i.e. from asymptomatic or symptomatic leaf tissue).

The recent observation of *C. nebraskensis* in ND has generated several questions on the pathogen in northern corn production. Therefore, the objectives of this study were to evaluate differences in aggressiveness among ND *C. nebraskensis* isolates and evaluate the genetic structure of the ND *C. nebraskensis* population.

Materials and Methods

Clavibacter nebraskensis **isolates**

Goss's leaf blight samples were collected from 2012 to 2017 during corn foliar disease surveys or obtained from submissions to the North Dakota State University Plant Diagnostic Laboratory. In some cases, multiple leaves were collected from fields with a high incidence of Goss's leaf blight. A total of 75 symptomatic leaves from plants exhibiting symptoms of the leaf blight phase of the disease were selected to represent the breadth of sampling years and ND corn producing regions and were given a unique identifier. Isolates included from surveys were named according to year of collection, field identification number, site within field (if

applicable), letter for each leaf (from one plant) collected, and number of distinct colonies isolated. For example, isolate 16-11.4-B #1 was collected in 2016, from the eleventh field scouted, in the fourth disease site in the field, from the second leaf collected, and was of a colony that differed in color and/or morphology from other colony(s) growing on the same *Corynebacterium nebraskense* selective (CNS) media plate (Gross and Vidaver 1979). Isolates submitted to the Diagnostic Laboratory are named for the year of submission, Diagnostic Laboratory catalog number, and the letter of the leaf in the sample. For example, 12-1504-A was submitted in 2012, its catalog number ends in 1504, and was isolated from the first leaf in the sample. Bacteria were extracted from infected leaf tissue using Agdia (Agdia, Inc., Elkhart, IN) mesh sample bags. Leaf tissue, between 6 and 8 cm^2 in size, was excised from the margin of healthy and infected tissue with a sterile razor blade and inserted between the mesh linings of a sample bag. Six milliliters of 10 mM potassium phosphate buffer (PB) were pipetted into each sample bag and leaf tissue was ground with a pen cap. Sample bags were immediately brought to the greenhouse for inoculation onto susceptible corn plants, Dekalb DKC37-38 (Monsanto Co., St. Louis, MO).

DKC37-38 was sown into PRO-MIX LP15 multi-purpose potting soil (Premier Tech Horticulture, Quakertown, PA) in 8.83L Elite 1000 nursery pots. Three seeds were sown per pot and pots were reduced to two plants approximately one week after planting. Pots received Micromax Micronutrients (ICL Specialty Fertilizers, Dublin, OH), containing 6% Ca and 3% Mg, and 14-14-16 Multicote 4 controlled release fertilizers at planting. Soil was watered with 21-7-7 Acid, Jack's Professional water-soluble fertilizer (JR Peters, Inc., Allentown, PA) beginning approximately 10 days after planting, then once a week until inoculation. Growing conditions were 22.2 to 24.4°C with plants receiving a 12-h photoperiod. Humidity was controlled between

85 and 90%. When plants had four to six leaves (V4 to V6), the top two to three leaves were inoculated with the extracted bacteria/phosphate buffer suspension. Disease lesions were allowed to progress for two to three weeks at which point, leaves were collected, dried, and stored for further bacterial isolation.

To obtain single colony isolates, a six to eight centimeter squared piece of symptomatic leaf tissue was excised and placed in an Agdia mesh sample bag. Three millimeters of PB were added and tissue was ground until translucent. Then, an inoculating loop was immersed into the sample bag and bacteria was streaked onto CNS media. Media was prepared according to Bergey's Manual of Systematic Bacteriology. Cultures were allowed to grow for 5 to 7 days at 21.1 to 23.9°C. Colony color, size, and morphology were recorded after 5 to 7 days on CNS. Colony color was categorized as either orange, yellow, light orange, pale orange, or a combination of colors denoted by "/". Colony sizes were classified as very small (1 mm), small (2-3 mm), or large (4-5 mm). Colony morphology was recorded as mucoidal or fluidal. A representative colony was selected and streaked onto nutrient broth yeast (NBY) media. Cultures were again allowed to grow for 5 to 7 days at 21.1 to 23.9°C before another single colony was selected and streaked onto a second plate of NBY. Single colony isolates from NBY were put into long-term storage on beads (Microbank Bacterial and Fungal Preservation System) at -80°C.

Pathogenicity assay

Single-colony isolates were grown on NBY prior to inoculation. Cultures were grown for 5 to 7 days at 21.1 to 23.9°C, flooded with 4 ml of PB, scraped into 15 ml centrifuge tubes, and diluted with PB to a final volume of 10 ml. The susceptible hybrid DKC37-38 was inoculated at the V4 to V6 growth stages in the greenhouse. Two leaves per plant, between the fourth and sixth leaves, were inoculated. Corn leaves were wetted with reverse osmosis water prior to

inoculation to increase humidity in the canopy. The pin-prick method was selected for inoculation (Calub et al. 1974). Modifications were made to the inoculating tool to fit the scope of greenhouse inoculations. The inoculating tool was modeled after the device described by Hagborg (1970). This consisted of a tongue-seizing forceps fitted with two rubber stoppers (Fig. 2.1). One stopper was uniformly fitted with three pins to create a wound in the leaf and an entry point for the bacteria (Fig 2.2). Pins were positioned in a straight line to provide a wound that was perpendicular to the midrib of the corn leaf. The second stopper was a gauged stopper into which a 1 ml syringe was inserted (Fig 2.3). Tubes were shaken to ensure bacteria was in suspension and 1 ml of bacteria suspension was drawn into the syringe. The syringe was then fitted into the gauged stopper and depressed forcing bacteria into the wound created by the pins. The plunger was depressed slowly, to the count of 8 s, to ensure bacteria entered the wound. Each corn plant was inoculated with one isolate. A new syringe was used for each isolate and forceps were sterilized with 70% EtOH between isolates. Isolates were deemed pathogenic if they produced lesions with water soaking and freckles characteristic of Goss's wilt and leaf blight. Disease lesions were allowed to progress for 21 days post-inoculation (dpi) and then leaves were collected, pressed, and dried for storage.

Figure 2.1. Modified tongue-seizing forceps used for the pin-prick inoculation method under greenhouse conditions.

Figure 2.2. Three pins fitted in a line across rubber stopper inserted into tongue-seizing forceps. Pins serve to wound corn leaves at the point of inoculation.

Figure 2.3. Gauged rubber stopper inserted into tongue-seizing forceps. A 1 ml syringe is fitted into the stopper to deliver *Clavibacter nebraskensis* inoculum into wounded corn leaves.

Aggressiveness assay

Forty-nine pathogenic isolates were selected for inclusion in an aggressiveness assay. For this study, aggressiveness is defined as the amount of disease in terms of lesion length produced over time. The reference isolate 'ND Cmn 2011', the original isolate identified in ND, was also included in the assay. The susceptible field corn hybrid DKC37-38 was inoculated under greenhouse conditions (described previously). *Clavibacter nebraskensis* isolates were grown on NBY, one Petri dish per replication. The aggressiveness experiment was conducted in the greenhouse with isolates appearing in a completely randomized design with three replications and then repeated. Each replicate consisted of one pot in which two plants were grown. Due to greenhouse constraints and time demands of inoculation, the 50 isolates were divided arbitrarily into three groups, with one group of isolates inoculated each day over a period of three consecutive days. Isolates' randomization into groups for the second run was independent of randomization in the first run. Disease assessments began at 4 days post-inoculation (dpi) and were repeated at 7, 10, 14, and 21 dpi. At each assessment date, total lesion length and total leaf

length were measured in mm on every inoculated leaf. Lesion length was measured from point of inoculation to the apex margin (apical) and from point of inoculation to the basal margin (basal). Width measurements included the expanse of the lesion at its widest point as well as one-half of the leaf width. Area under the disease progress curve (AUDPC) was calculated for basal, apical, and total lesion lengths. This was done using the AUDPC formula (Shaner and Finney 1977):

$$\sum_{i=1}^{n} [(Y_{i+1} + Y_i)/2] [X_{i+1} - X_i]$$

where Y_i = percentage disease severity at the i*th* observation and X_i = time in days at the i*th* observation. At 21 dpi, inoculated leaves were labeled, then cut at the leaf attachment, pressed, and dried overnight. The following day, leaf surface area was measured on a LI-3100C scanning area meter with conveyor belt (LI-COR Biosciences, Inc., Lincoln, NE). When leaves were fully dry, the diseased portion of each leaf was excised and measured on the LI-3100C area meter. Proportion infected leaf area was calculated by dividing the infected tissue area by one-half of total leaf area.

Genotypic assay

DNA extraction

Genomic DNA was isolated using a MP Biomedicals FastDNA SPIN KIT (MP Biomedicals, Santa Ana, CA). Modifications were made to the manufacturer's protocol for optimization. Isolates of *C. nebraskensis* were grown for 48 to 72 h in nutrient broth at 21.1 to 23.9°C under constant florescent light on a VWR 3500 Advanced digital orbital shaker table (VWR International, LLC, Radnor, PA) set to 150 rpm. A total of 4 to 5 ml of broth culture were centrifuged at 14,000 x g for 15 min to pellet bacterial cells. A total wet weight of 50 to 100 mg was resuspended in 200 µl sterile distilled water and used for DNA extraction. To begin extraction, the sample and 1.0 ml of Cell Lysis Solution (CLS-TC) were added to Lysing Matrix

A tube. Lysing Matrix A tubes were shaken by hand for 40 s. The tubes were then centrifuged at

14,000 x g for 15 min to pellet debris. The supernatant, at a volume of 800 µl, was transferred to

a 2.0 ml microcentrifuge tube and an equal volume of Binding Matrix was added. Tubes were

inverted to mix the sample and Binding Matrix. An incubation period of 10 min followed. Tubes

were placed on a rotator at room temperature for the duration of the incubation. A total of 600 µl

of the suspension were transferred to a SPIN Filter and centrifuged at 14,000 x g for 2 min. The

contents of each catch tube were discarded and filters were replaced in catch tubes. Another 600

µl of the suspension was added to the SPIN Filter and centrifuged for 2 min. The filtration step

was completed a total of three times and tubes were shaken each time to resuspend any

sediments at the bottom of the tube. SEWS-M was then added to the filter and the pellet was

resuspended with the force of the pipette tip. Centrifugation followed at 14,000 x g for 2 min.

The contents of the tube were discarded and the centrifugation repeated. At this point, the filters

were placed in clean catch tubes and DNA was eluted by adding 50 µl of DES buffer. Tubes

were incubated in a water bath set to 55°C for 5 min. Then, a final centrifugation at 14,000 x g

for 1.5 min was performed. DNA was stored at 4°C or -20°C until library preparation for

genotyping-by-sequencing (GBS).

Library preparation and bioinformatic analyses

Approximately 100 ng of genomic DNA from each bacterial sample was used to generate

sample-specific barcoded whole-genome shotgun libraries using NEBNext Fast DNA

Fragmentation and Library Prep Set for Ion Torrent (New England Biolabs, Inc., Ipswich, MA).

The barcoded libraries were multiplexed and sequenced using an Ion 540 chip in an Ion S5

sequencing system (Ion Torrent Systems, Inc., Gilford, NH). The sequencing reads were

trimmed for quality using default settings in CLC Genomics Workbench 8 (QIAGEN, Hilden,

Germany).

The quality trimmed reads were mapped to a *Clavibacter nebraskensis* reference genome, NCPPB 2581 (NCBI accession NC_020891) using the Burrows-Wheeler Aligner maximal exact match (BWA-MEM) algorithm (Li 2013). The mapped reads were tagged for PCR duplicates using the MarkDuplicates function of Picard tools (accessed at http://broadinstitute. github.io/picard). Finally, variants (single nucleotide polymorphisms (SNPs) and insertions/deletions (INDELs)) were called using the GATK HaplotypeCaller tools in ERC GVCF mode with the parameters suggested for genomic sequences (Van der Auwera et al. 2013). The individual g.vcf files were combined using GATK GentoypeGVCFs tool to generate final variant call format (VCF) files containing variants from all samples. Each individual variant call per sample was filtered for the variants with genotype quality greater than 10 and read depth greater than 4 using Vcftools (Danecek et al. 2011). The variants with minor allele frequency (MAF) less than 5% and missing data greater than 40% were removed for analysis. The allele frequency of variants per sample was corrected for heterozygous calls using a custom visual basic script to generate a final VCF file for subsequent analysis (Sharma Poudel, 2018).

Statistical analyses

Aggressiveness assay analyses

Prior to analysis, square root transformations were used on lesion lengths to normalize data. The dependent variables analyzed included total lesion length (TLL), basal lesion length (BLL), and apical lesion length (ALL) at 4 and 21 dpi. In addition, final AUDPC values (aggressiveness) for TLL, BLL, and ALL were analyzed. Isolates considered failures (i.e. infection did not take across all replicates of the isolate in either repetition) were not included in statistical analyses. Data were analyzed with the general linearized mixed model (GLIMMIX) analysis of variance using Statistical Analysis Software (SAS) (v. 9.4; SAS Institute, Cary, NC). Replicate and repetition (run) were treated as random effects and were combined as a nested

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random effect [rep(run)]. Isolate was treated as a fixed effect. Least squares means were back-transformed and presented in the results. A *t*-test comparison was used to separate isolates into three aggressiveness categories; most, intermediate and least aggressive. Specifically, the AUDPC value for each isolate was compared to the isolate with highest and lowest value. PROC CORR in SAS was used to calculate the Pearson correlation coefficient and to test for a linear relationship between lesion proportions for values obtained through physical measurements at 21 dpi and values obtained with the LI-COR area meter.

Population genetic analyses

The filtered and corrected VCF files were converted using the statistical software R (R Foundation for Statistical Computing, Vienna, Austria) into R-packages *poppr* (Kamvar et al. 2014; 2015) and adgenet (Jombart et al. 2008) readable formats using R-package *vcfR* (Knaus and Grünwald 2017). A distance tree was constructed using the unweighted pair group method with arithmetic mean (UPGMA) algorithm with 100 bootstrapping. The structure/variance in the population based on region (North Dakota agricultural statistics districts, NASS 2018) and years (Figure 2.4) were inferred using principal component analysis (PCA) and discriminant analysis of principal components (DAPC). Composite plots were generated to assess and visualize the admixture in samples collected from different regions and years.
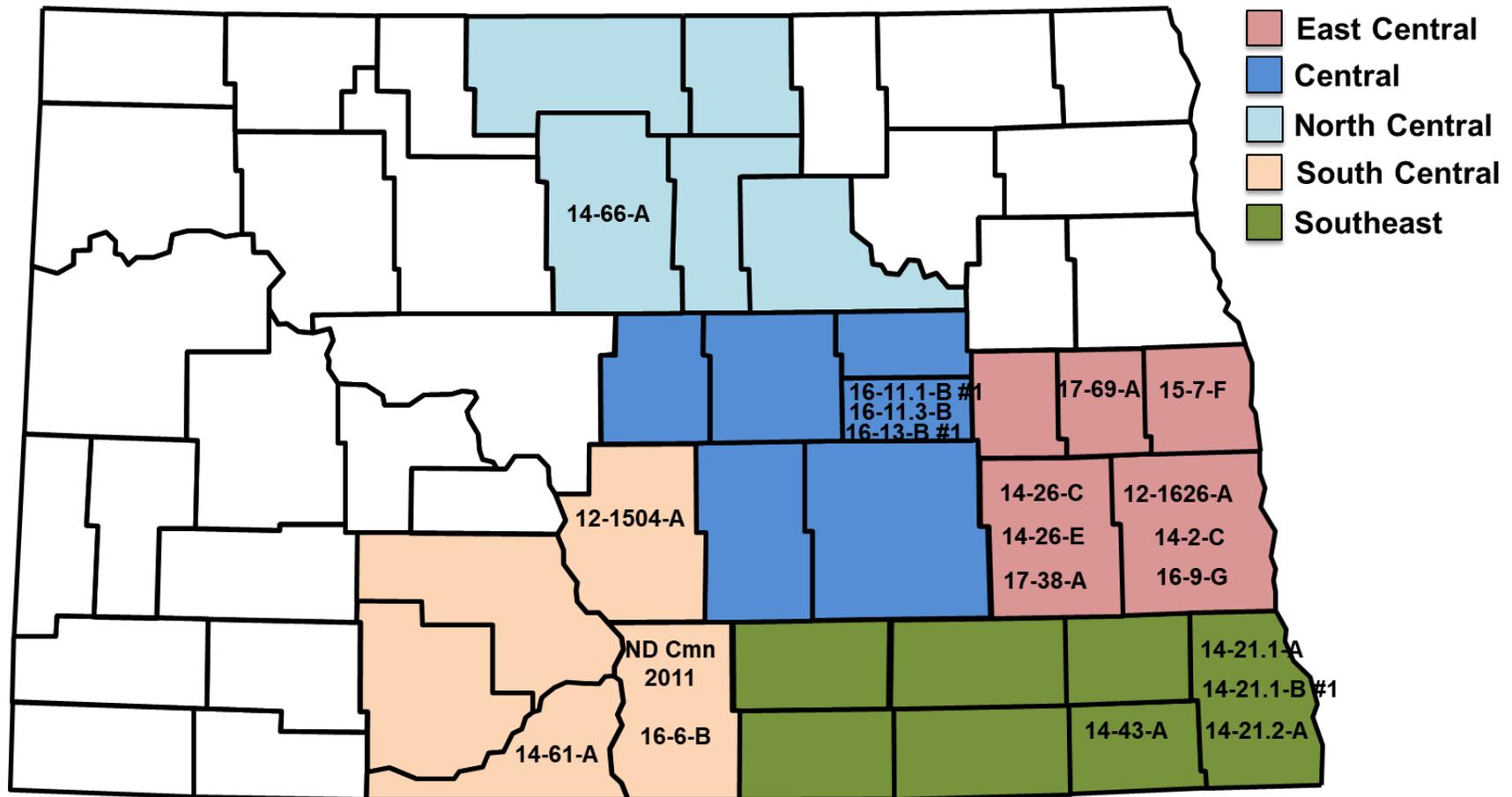
Figure 2.4. North Dakota agricultural statistics districts (regions) represented by the 20 ND *Clavibacter nebraskensis* isolates. Regions are colored on the map as indicated in the legend and isolates are listed in their county of origin.

Results

Pathogenicity assay and colony characteristics

Sixty-two of 98 isolates examined were found to be pathogenic (Table 2.1) with water soaking symptoms evident as early as five dpi. Colony size differences were apparent and 3, 86, and 9 isolates were categorized as very small, small, and large, respectively. Most of isolates were further defined as mucoidal (93 isolates) and only 5 isolates were defined as fluidal. Colony colors were yellow and shades of orange with a majority of isolates being orange.

Apical lesion length

Significant differences in ALL were observed among isolates at each assessment (Table 2.2). The range of measurements for ALL at 4 dpi was 0 to 4.2 mm with isolate 14-43-A having a significantly higher value than 86% of the isolates. At 21 dpi, ALL measurements ranged from 1.8 to 36.8 mm. Isolate 16-11.1-B #1 had the highest ALL after 21 days, which was statistically higher than 68% of the isolates.

Basal lesion length

Significant differences in BLL were also observed among isolates at each assessment (Table 2.3). With some exceptions, most of the BLL measurements for each isolate were lower than the ALL. At 4 dpi, BLL measurements ranged from 0 to 1.6 mm and isolate 14-37-B had the largest lesion at 1.6 mm, which was statistically higher than 82% of the isolates. At 21 dpi, lesion length varied from 0.5 to 18.2 mm. Isolate 16-6-B had the largest BLL, which was statistically higher than 80% of the isolates.

Table 2.1. Pathogenicity assay results and morphological characterization of North Dakota *Clavibacter nebraskensis* isolates obtained from 2012 to 2017.

Year	Isolate	Pathogenic	Included[y]	Colony morphology[z]	Colony color
2012	12-1504-A	Yes	*	Small mucoid	Orange
2012	12-1504-B	Yes		Small mucoid	Orange
2012	12-1528-B	Yes	*	Small mucoid	Orange
2012	12-1553-A	Yes	*	Small mucoid	Orange
2012	12-1626-A	Yes	*	Small mucoid	Orange
2013	13-1134-A	No		Small mucoid	Orange
2013	13-1135-A	Yes	*	Small mucoid	Orange
2014	14-2-C	Yes	*	Small mucoid	Orange
2014	14-11-D	Yes		Small mucoid	Orange
2014	14-21.1-A	Yes	*	Small mucoid	Orange
2014	14-21.1-B #1	Yes	*	Small mucoid	Orange
2014	14-21.1-B #2	No		Small mucoid	Yellow
2014	14-21.1-J	No		Small mucoid	Orange
2014	14-21.2-A	Yes	*	Small mucoid	Orange
2014	14-21.2-B	Yes		Small mucoid	Orange
2014	14-23-B	Yes	*	Small mucoid	Orange
2014	14-23-C	No		Small mucoid	Orange
2014	14-26-C	Yes	*	Small mucoid	Orange
2014	14-26-E	Yes	*	Small mucoid	Orange
2014	14-37-B	Yes	*	Small mucoid	Orange
2014	14-37-C	No		Small mucoid	Orange
2014	14-39-K	Yes	*	Small mucoid	Orange
2014	14-39-N	Yes		Small mucoid	Orange
2014	14-43-A	Yes	*	Small mucoid	Orange
2014	14-56-B	Yes	*	Small mucoid	Orange
2014	14-58-A	Yes	*	Small mucoid	Orange
2014	14-58-B	Yes	*	Small mucoid	Orange
2014	14-61-A	Yes	*	Small mucoid	Orange
2014	14-62-A	Yes		Small mucoid	Orange
2014	14-64-C	Yes	*	Small mucoid	Orange
2014	14-66-A	Yes	*	Small mucoid	Orange
2014	14-66-B	Yes		Small mucoid	Orange
2015	15-3-B	Yes	*	Small mucoid	Orange
2015	15-3-C	Yes		Small mucoid	Orange
2015	15-7-B #1	No		Small mucoid	Orange
2015	15-7-B #2	No		Large mucoid	Orange
2015	15-7-B #3	No		Small mucoid	Yellow
2015	15-7-F	Yes	*	Small mucoid	Orange
2015	15-28-A	Yes	*	Small mucoid	Orange
2015	15-28-C #1	Yes	*	Small mucoid	Light orange
2015	15-28-C #2	Yes		Small mucoid	Orange
2015	15-29-A	Yes	*	Small mucoid	Orange
2016	16-1-D	Yes		Small mucoid	Orange
2016	16-2-B	Yes	*	Small mucoid	Orange
2016	16-3-B	Yes		Small mucoid	Orange
2016	16-4-A	Yes	*	Small mucoid	Orange
2016	16-5-A	Yes	*	Small mucoid	Orange
2016	16-6-B	Yes	*	Small mucoid	Orange
2016	16-9-G	Yes	*	Small mucoid	Orange
2016	16-11.1-A	Yes	*	Small mucoid	Orange
2016	16-11.1-B #1	Yes	*	Small mucoid	Orange
2016	16-11.1-B #2	No		Large mucoid	Orange

Table 2.1. Pathogenicity assay results and morphological characterization of North Dakota *Clavibacter nebraskensis* isolates obtained from 2012 to 2017 (continued).

Year	Isolate	Pathogenic	Included[y]	Colony morphology[z]	Colony color
2016	16-11.1-D	Yes	*	Small mucoid	Orange
2016	16-11.2-A	No		Small mucoid	Orange
2016	16-11.2-B #1	No		Small mucoid	Orange
2016	16-11.2-B #2	Yes	*	Very small mucoid	Orange
2016	16-11.3-A #1	No		Very small mucoid	Orange
2016	16-11.3-A #2	No		Large fluidal	Orange
2016	16-11.3-A #3	No		Small mucoid	Orange
2016	16-11.3-B	Yes	*	Small mucoid	Orange
2016	16-11.4-A #1	Yes	*	Small mucoid	Light orange
2016	16-11.4-A #2	No		Small mucoid	Yellow/orange
2016	16-11.4-B	Yes	*	Small mucoid	Orange
2016	16-12-A	Yes	*	Small mucoid	Pale orange
2016	16-13-B #1	Yes	*	Small mucoid	Orange
2016	16-13-B #2	No		Small mucoid	Yellow
2016	16-28-A #1	No		Small mucoid	Yellow
2016	16-28-A #2	No		Large fluidal	Orange
2016	16-35-A #1	No		Small mucoid	Orange
2016	16-35-A #2	No		Large mucoid	Orange
2016	16-84-D	Yes	*	Small mucoid	Orange
2017	17-9-A	Yes		Small mucoid	Orange
2017	17-9-B #1	No		Small mucoid	Yellow
2017	17-9-B #2	No		Large fluidal	Orange
2017	17-9-B #3	Yes	*	Small mucoid	Orange
2017	17-23-A #1	Yes		Small mucoid	Orange
2017	17-23-A #2	No		Large mucoid	Orange
2017	17-27-A #1	No		Small mucoid	Yellow
2017	17-27-A #2	No		Small mucoid	Orange
2017	17-27-A #3	No		Large fluidal	Orange
2017	17-30-A	No		Small mucoid	Orange
2017	17-31-A #1	No		Small mucoid	Orange
2017	17-31-A #2	No		Small mucoid	Yellow
2017	17-31-B #1	No		Small mucoid	Yellow
2017	17-31-B #2	Yes	*	Small mucoid	Orange
2017	17-38-A	Yes	*	Small mucoid	Pale Orange
2017	17-43-A	No		Small mucoid	Orange
2017	17-43-B	Yes	*	Small mucoid	Orange
2017	17-47-A	Yes	*	Small mucoid	Orange
2017	17-49-A #1	No		Small mucoid	Yellow
2017	17-49-A #2	No		Small mucoid	Orange
2017	17-66-A #1	No		Small mucoid	Yellow
2017	17-66-A #2	Yes	*	Small mucoid	Orange
2017	17-69-A	Yes	*	Small mucoid	Orange
2017	17-81-A #1	No		Small mucoid	Orange
2017	17-81-A #2	Yes		Very small mucoid	Orange
2017	17-99-A #1	Yes	*	Small mucoid	Orange
2017	17-99-A #2	No		Large fluidal	Orange

[y]Included (*) isolates were used in the greenhouse *Clavibacter nebraskensis* aggressiveness assay.
[z]Colony size was measured after 5 to 7 days of growth on nutrient broth yeast media. Colony size was classified as very small (1 mm) in diameter, small (2-3 mm), or large (4-5 mm).

Table 2.2. Least square mean estimates of lesion lengths from the point of inoculation to the apex (ALL) for North Dakota *Clavibacter nebraskensis* isolates at 4, 7, 10, 14, and 21 days post-inoculation.

Code[x]	Isolate	Lesion length (mm)				
		4 dpi[y]	7 dpi	10 dpi	14 dpi	21 dpi
10	14-43-A	4.2	13.7	20.0	24.3	28.6
8	14-37-B	3.4	13.5	20.8	26.8	28.8
28	16-11.1-B #1	3.3	11.4	18.5	28.0	36.8
44	17-99-A #1	2.6	12.2	20.5	27.7	35.6
26	16-9-G	2.6	11.4	20.2	26.2	33.9
3	14-21.1-B #1	2.5	12.3	19.5	23.9	28.9
11	14-56-B	2.4	10.1	15.3	19.6	24.3
16	14-66-A	2.4	11.9	20.6	28.0	33.6
15	14-64-C	2.2	9.5	19.1	25.2	29.9
25	16-6-B	1.8	9.2	18.1	25.7	34.5
6	14-26-C	1.7	8.2	14.3	22.8	28.2
21	15-29-A	1.7	8.5	16.2	22.3	28.0
24	16-5-A	1.6	9.5	15.2	21.7	25.3
14	14-61-A	1.6	7.2	13.3	20.2	30.1
1	14-2-C	1.4	5.3	11.2	15.5	21.0
5	14-23-B	1.3	6.1	14.3	22.2	26.6
18	15-7-F	1.3	8.6	13.0	16.2	21.0
30	16-11.2-B #2	1.0	5.1	12.4	18.0	24.6
13	14-58-B	1.0	6.0	11.5	15.8	21.9
49	13-1135-A	0.9	3.6	6.9	10.7	16.3
22	16-2-B	0.8	7.2	10.5	15.2	18.8
31	16-11.3-B	0.6	2.8	7.1	13.5	20.9
48	12-1626-A	0.6	1.9	4.2	7.4	11.0
43	17-69-A	0.6	2.1	4.0	6.2	9.6
45	12-1504-A	0.5	2.6	5.7	8.3	10.2
20	15-28-C #1	0.5	1.7	4.3	6.5	9.7
33	16-11.4-B	0.5	3.3	9.3	18.9	29.0
42	17-66-A #2	0.4	1.5	3.5	8.2	21.0
27	16-11.1-A	0.4	2.5	3.5	4.6	5.9
12	14-58-A	0.3	2.1	4.3	6.8	9.3
4	14-21.2-A	0.3	2.2	4.3	9.1	13.4
7	14-26-E	0.3	1.5	3.0	5.2	8.4
23	16-4-A	0.3	1.9	5.5	9.3	17.4
19	15-28-A	0.3	1.6	3.3	6.9	16.2
29	16-11.1-D	0.3	2.3	5.4	9.1	12.1
32	16-11.4-A #1	0.3	2.1	5.2	12.8	28.2
9	14-39-K	0.3	3.3	9.6	15.8	26.4
47	12-1553-A	0.3	2.4	6.0	7.8	11.4
35	16-13-B #1	0.3	2.8	5.9	10.2	18.0
37	17-9-B #3	0.0	0.3	0.7	1.4	1.8
41	17-47-A	0.0	3.5	9.0	17.4	22.8
38	17-31-B #2	0.0	0.1	0.9	1.8	2.4
39	17-38-A	0.0	0.1	0.3	1.1	1.5
46	12-1528-B	0.0	0.0	0.3	0.8	1.8
	P-value[z]	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[x]Code = the shorthand identification of isolates used for statistical analyses.

[y]dpi = days post-inoculation.

[z]Level of significance (*P*-value) for *t*-test comparisons ($\alpha = 0.05$).

Table 2.3. Least square mean estimates of lesion lengths from the point of inoculation to the base (BLL) for North Dakota *Clavibacter nebraskensis* isolates at 4, 7, 10, 14, and 21 days post-inoculation.

Code[x]	Isolate	Lesion length (mm)				
		4 dpi[y]	7 dpi	10 dpi	14 dpi	21 dpi
8	14-37-B	1.6	4.7	8.6	12.1	16.3
10	14-43-A	1.5	4.4	7.0	11.0	14.8
28	16-11.1-B #1	1.4	3.5	6.9	10.6	17.4
26	16-9-G	1.4	3.5	6.8	11.0	15.7
44	17-99-A #1	1.1	4.1	7.5	11.7	17.3
11	14-56-B	1.0	2.9	5.0	7.1	10.6
3	14-21.1-B #1	1.0	4.4	8.5	12.2	15.7
15	14-64-C	1.0	4.0	5.8	9.3	14.1
16	14-66-A	0.9	3.9	6.7	11.9	18.1
14	14-61-A	0.8	2.6	4.5	7.3	10.7
21	15-29-A	0.7	2.9	5.0	7.7	12.1
6	14-26-C	0.7	2.1	4.2	6.9	11.8
24	16-5-A	0.6	2.9	5.0	7.3	11.0
1	14-2-C	0.6	1.9	2.8	4.3	6.7
25	16-6-B	0.5	3.0	7.0	12.0	18.2
22	16-2-B	0.5	1.5	2.7	3.9	5.4
30	16-11.2-B #2	0.4	1.6	3.6	5.2	9.2
13	14-58-B	0.4	1.8	2.5	4.2	5.7
18	15-7-F	0.4	2.1	3.9	6.7	9.4
5	14-23-B	0.3	2.0	4.3	7.2	11.6
49	13-1135-A	0.3	1.0	2.0	3.2	6.5
43	17-69-A	0.3	0.7	1.3	1.9	4.2
45	12-1504-A	0.2	0.6	1.0	2.0	3.0
7	14-26-E	0.2	0.9	1.1	1.4	2.9
27	16-11.1-A	0.2	0.4	0.6	1.6	2.3
48	12-1626-A	0.2	0.7	1.1	2.1	4.5
33	16-11.4-B	0.2	0.9	2.4	4.8	9.4
35	16-13-B #1	0.2	0.8	1.7	3.5	7.4
4	14-21.2-A	0.2	0.6	1.5	3.2	6.2
20	15-28-C #1	0.2	0.2	0.4	1.5	2.8
47	12-1553-A	0.2	0.6	1.2	2.3	3.7
9	14-39-K	0.1	0.4	1.1	3.0	6.2
31	16-11.3-B	0.1	0.3	1.3	1.5	5.1
42	17-66-A #2	0.1	0.4	0.8	2.1	5.7
29	16-11.1-D	0.1	0.4	0.8	1.6	2.7
12	14-58-A	0.1	0.5	0.8	1.7	2.9
23	16-4-A	0.1	0.5	0.9	2.4	5.6
32	16-11.4-A #1	0.1	0.0	0.5	1.3	10.0
41	17-47-A	0.1	0.8	1.9	4.2	6.8
19	15-28-A	0.0	0.2	0.4	1.5	5.3
37	17-9-B #3	0.0	0.1	0.1	0.5	0.7
38	17-31-B #2	0.0	0.0	0.2	0.4	1.0
39	17-38-A	0.0	0.0	0.1	0.4	0.7
46	12-1528-B	0.0	0.0	0.0	0.1	0.5
	P-value[z]	< 0.0001	< 0.0001	< 0.0001	< 0.0001	< 0.0001

[x]Code = the shorthand identification of isolates used for statistical analyses.
[y]dpi = days post-inoculation.
[z]Level of significance (*P*-value) for *t*-test comparisons (α = 0.05).

AUDPC (Aggressiveness)

AUDPC values for TLL ranged from 17 to 571. The largest AUDPC value was for isolate 17-99-A #1 which was considered the most aggressive isolate. The least aggressive isolate was 12-1528-B. Using the most and least aggressive isolates, comparative *t*-tests resulted in three groupings: Group A contained isolates that were statistically similar to the most aggressive isolate; Group B isolates were statistically different from both the most and least aggressive isolate; and Group C included isolates that were statistically similar to the least aggressive isolate. Results of the *t*-test indicated 14 other isolates grouped with 17-99-A #1 (Group A; most aggressive); 25 isolates were classified into Group B (intermediately aggressive) and 3 other isolates were placed in Group C with the least aggressive isolate 12-1528-B (Figure 2.5).
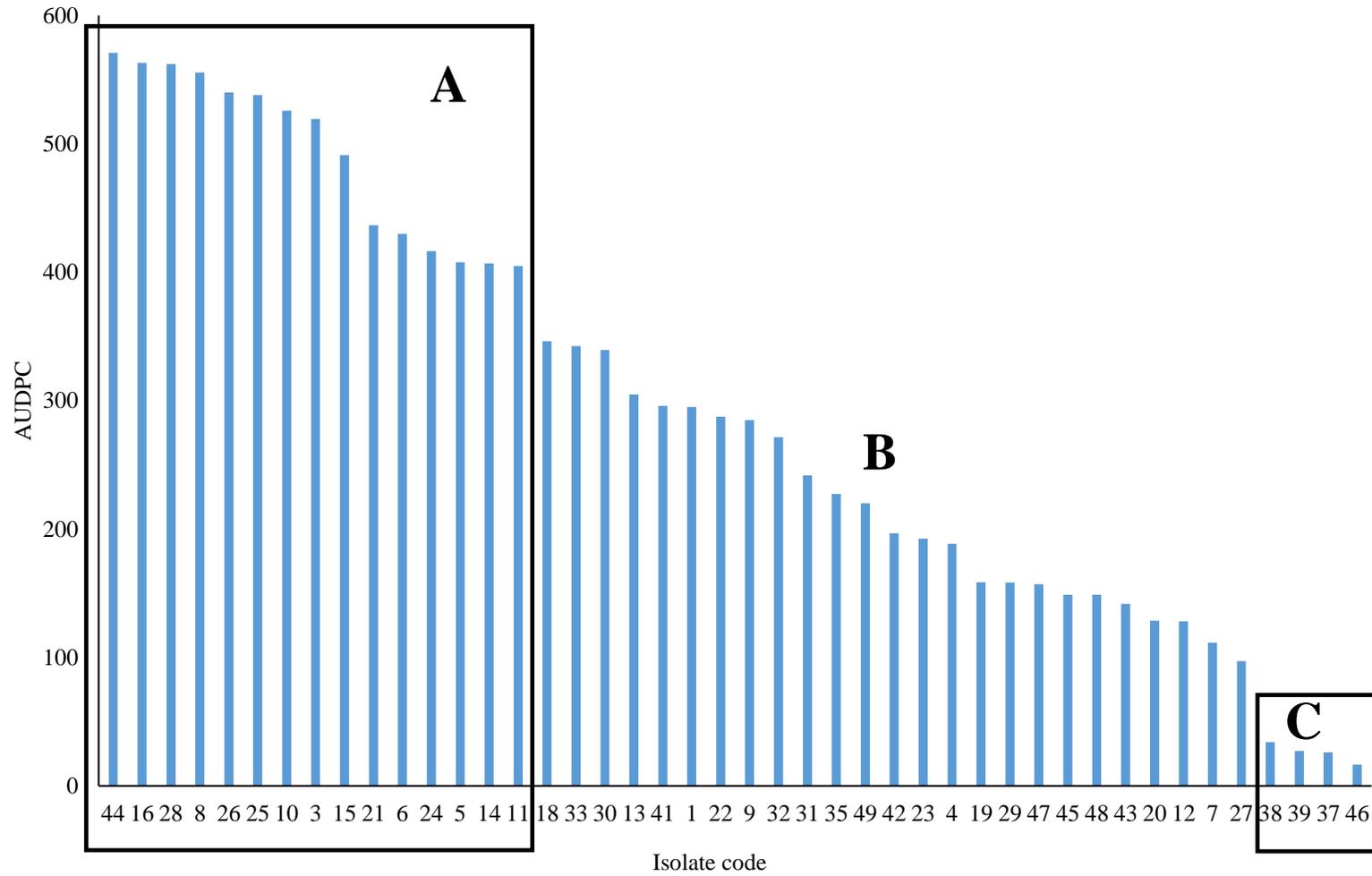
Figure 2.5. Area under the disease progress curve for total lesion length of North Dakota *Clavibacter nebraskensis*. Aggressiveness groupings indicated by A, B, or C based on *t*-tests at *P* = 0.05.

Differing levels of aggressiveness were observed among isolates collected from the same field. Isolates 14-21.1-B #1 and 14-21.2-A were classified in Group A and Group B, respectively. Similarly, 14-26-C and 14-26-E had different levels of aggressiveness, as the former belonged to Group A and the latter to Group B. Six of the seven isolates from field 16-11 were classified as moderately aggressive (Group B), while one isolate, 16-11.1-B #1, classified as most aggressive (Group A).

Correlation of measurements

The value of using a leaf area predictive scanner was evaluated 21 dpi. When comparing lesion proportions from the LI-COR area meter measurements and lesion proportions from physical measurements at 21 dpi, a significant linear relationship existed ($P = < 0.0001$) (Figure 2.6). The coefficient of determination for the linear model was 0.73.

Figure plot: x-axis "LI-COR lesion proportion", y-axis "21 dpi lesion proportion". Equation shown on plot:

$$y = 1.0538x + 0.0204$$
$$R^2 = 0.7268$$
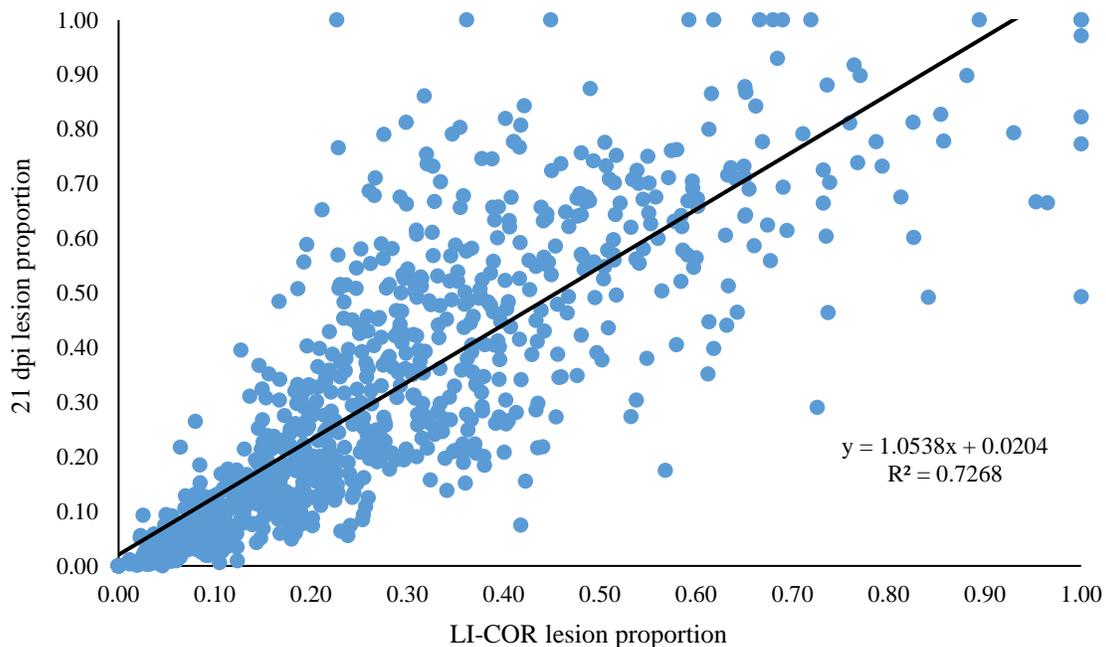
Figure 2.6. Correlation of lesion proportion measured mechanically on a LI-COR LI-3100C scanning area meter and lesion proportion measured manually at 21 days post-inoculation (dpi). The coefficient of determination (R^2) was 0.73.

Genotypic assay

A total of 37M single end reads (average of 1.7M S.D. ±1.7M reads per sample) with an average read length of 174bp were obtained for 22 samples (Table 2.4). In total, 15451 raw variants were obtained, which after filtering for quality, missing data, and MAF resulted in 4170 variants. Two additional samples were removed due to missing data greater than 90% and thus, 20 samples were used in the population genetic analyses.

Table 2.4. *Clavibacter nebraskensis* isolates included in genetic analyses.

Year	Isolate	Region[z]	Missing data (%)	Trimmed reads
2011	ND Cmn 2011	SC	1	4102833
2012	12-1504-A	SC	1	1425001
2012	12-1626-A	EC	0	5542003
2014	14-2-C	EC	1	1728190
2014	14-21.1-A	SE	1	1530908
2014	14-21.1-B #1	SE	1	1077551
2014	14-21.2-A	SE	1	1051528
2014	14-26-C	EC	4	5319648
2014	14-26-E	EC	2	645637
2014	14-43-A	SE	4	455104
2014	14-61-A	SC	1	2915312
2014	14-66-A	NC	2	4278649
2015	15-7-F	EC	19	207904
2016	16-6-B	SC	1	1380997
2016	16-9-G	EC	5	428149
2016	16-11.1-B #1	C	24	199563
2016	16-11.3-B	C	3	559577
2016	16-13-B #1	C	1	3099267
2017	17-38-A	EC	2	644816
2017	17-69-A	EC	13	258694

[z]North Dakota agricultural statistics districts (regions): EC = East Central, C = Central, NC = North Central, SC = South Central, and SE = Southeast.

The genetic distance tree (Figure 2.7) indicated genetic differences exist in the ND *C. nebraskensis* population, however the population did not cluster based on isolate origin (region) or year of isolation. Isolates from fields 14-26 and 14-21 were placed close to one another and were connected by short branches. Samples from different pockets within field 14-21 (14-21.1 and 14-21.2) were separated by an additional short branch. With principal components analysis (PCA), two components were retained (PC1 and PC2) as they explained 62% of the variation in the data set. PC1, which by itself accounted for the majority of the variation, was unable to separate the samples into distinct groups based on either region or year (Figures 2.8 and 2.9). This was evidenced by the overlapping of ellipses. The DAPC plots showed similar results and are presented in Appendices B.2 and B.3. The composite plot for assigned populations by year was skewed as the majority of the 20 isolates were collected in 2014 (data not shown). The composite plot for assigned populations by region showed admixture among isolates (Figure 2.10). Most isolates were assigned to all regions and had moderate to high probability of belonging to regions other than what they originated from.
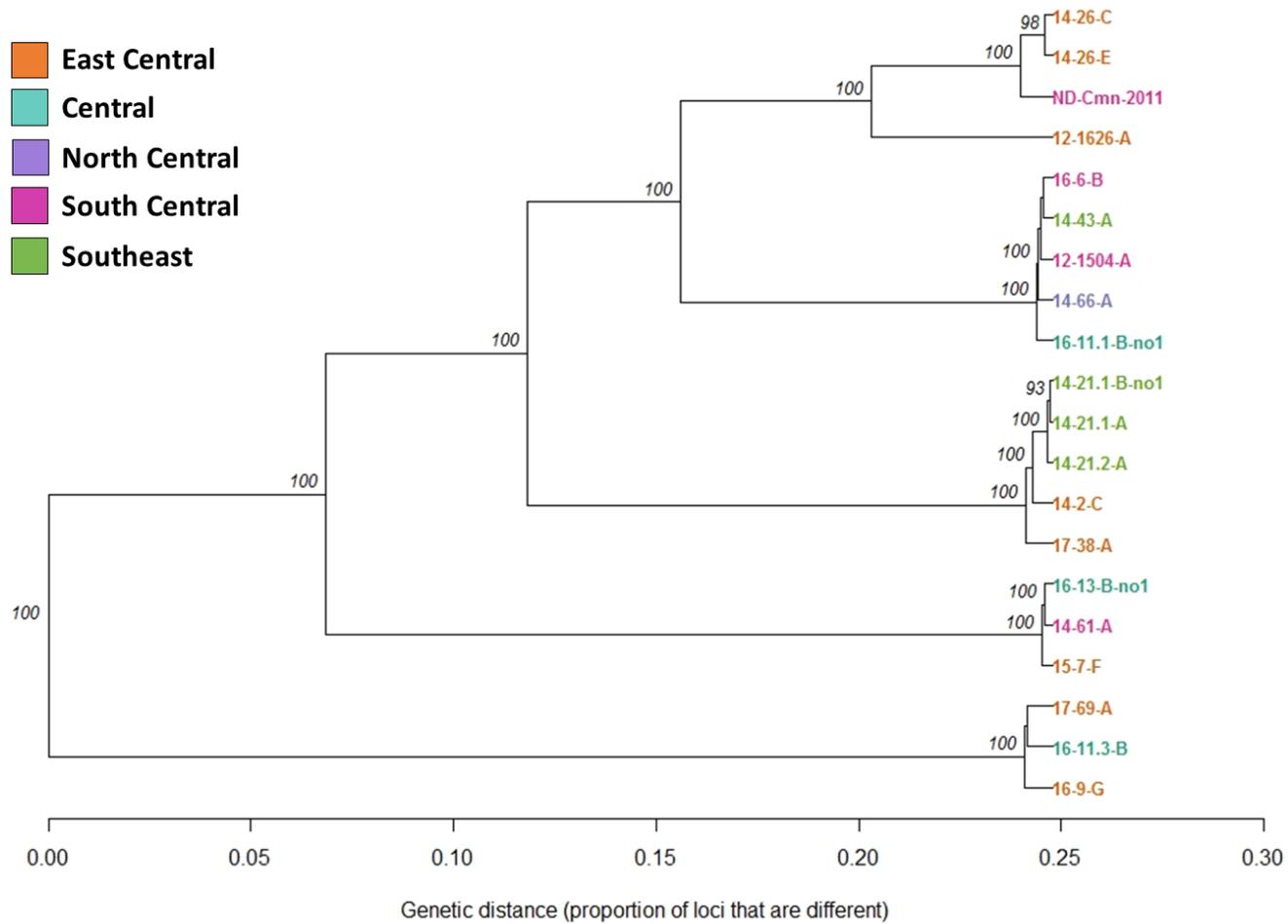
Figure 2.7. Genetic distance tree based on the unweighted pair group method with arithmetic mean algorithm and constructed with 100 bootstrapping using 20 *Clavibacter nebraskensis* isolates. Isolates are colored according to North Dakota agricultural statistics districts (regions), which are presented in the legend.
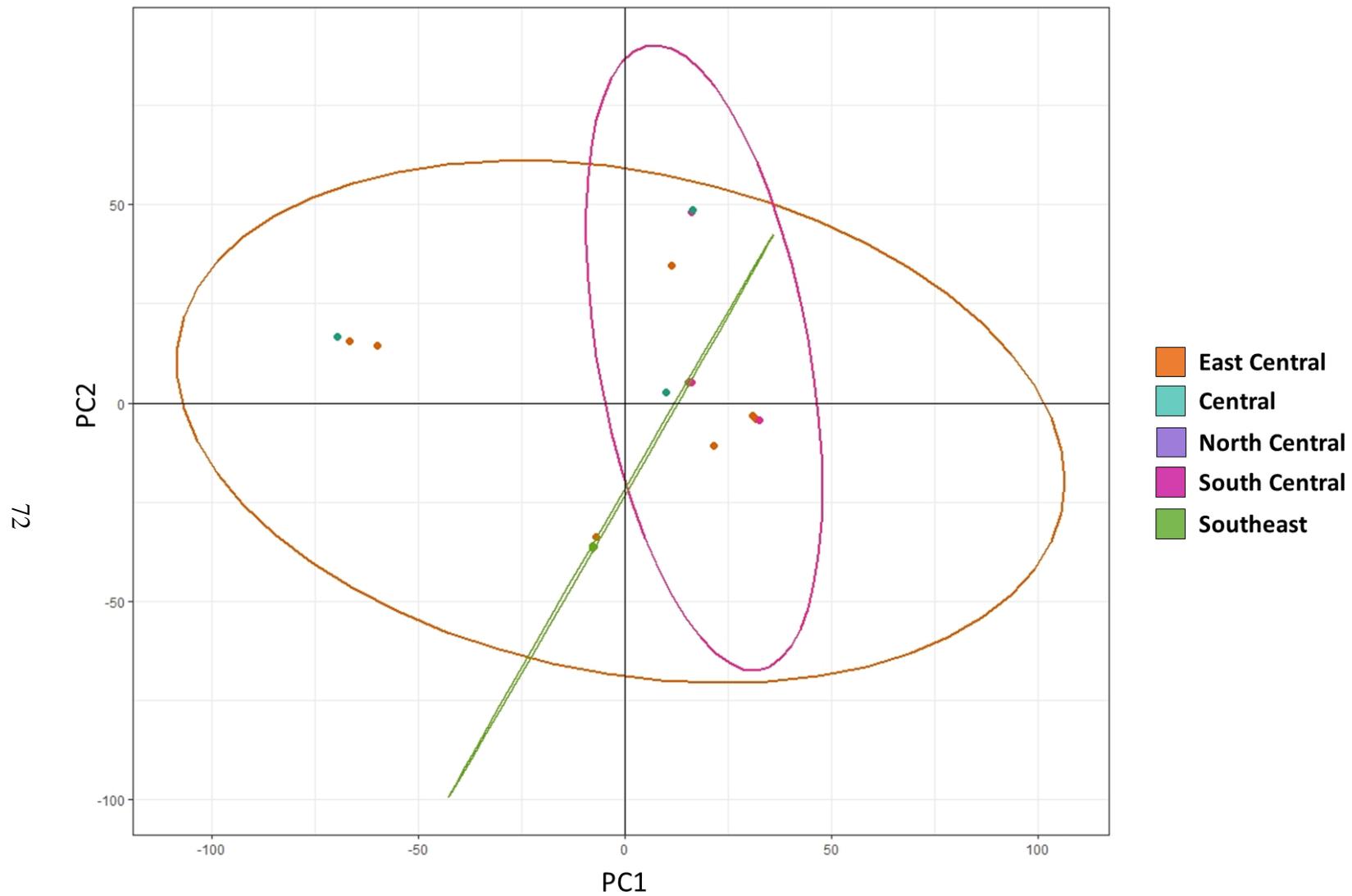
Figure 2.8. Principal components analysis of SNP data for 20 North Dakota (ND) *Clavibacter nebraskensis* isolates. Isolates are colored according to their ND agricultural statistics district (region) of origin. Regions are presented in the legend.
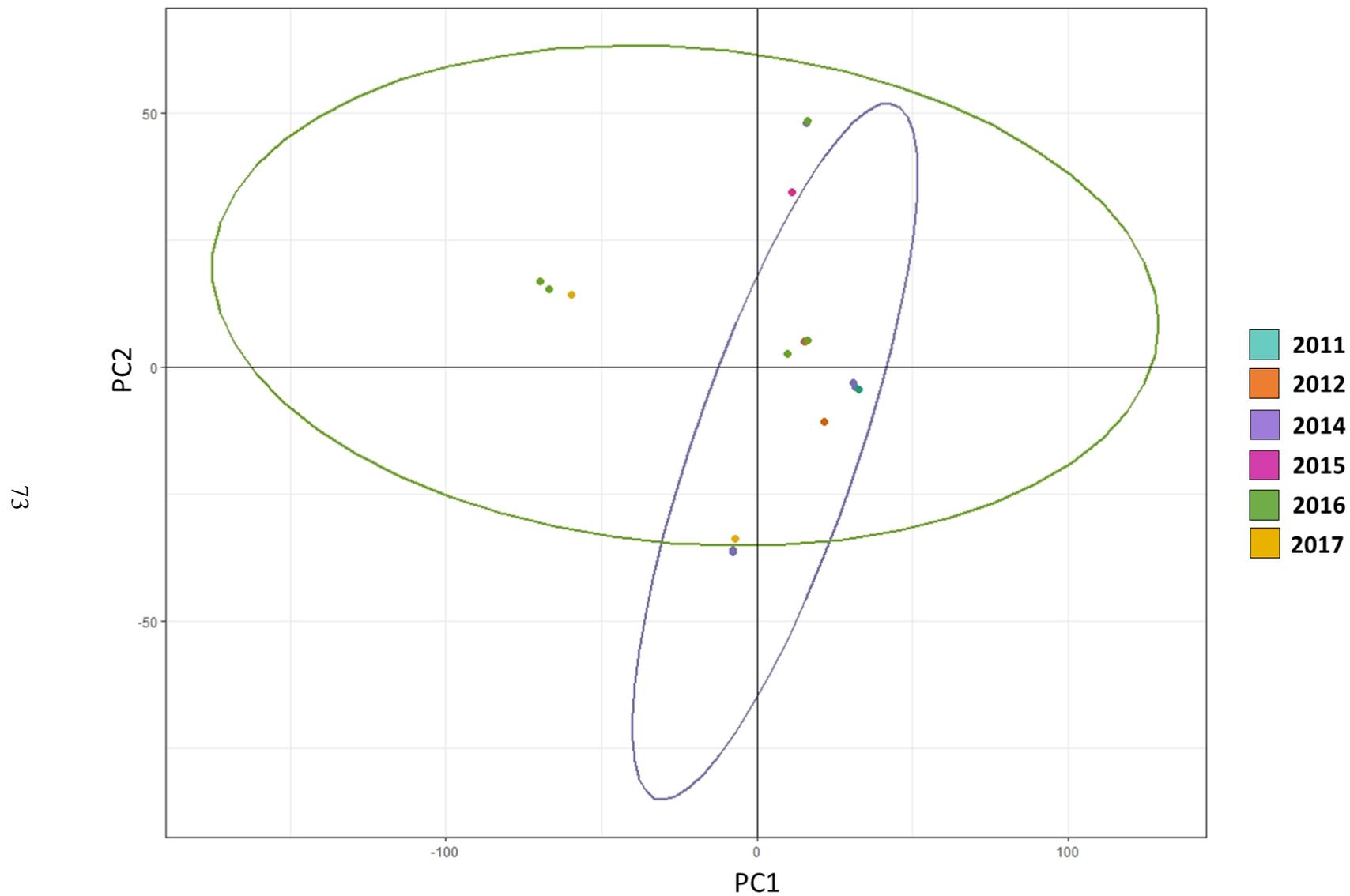
Figure 2.9. Principal components analysis of SNP data for 20 North Dakota *Clavibacter nebraskensis* isolates. Isolates are colored by their year of collection, which are presented in the legend.
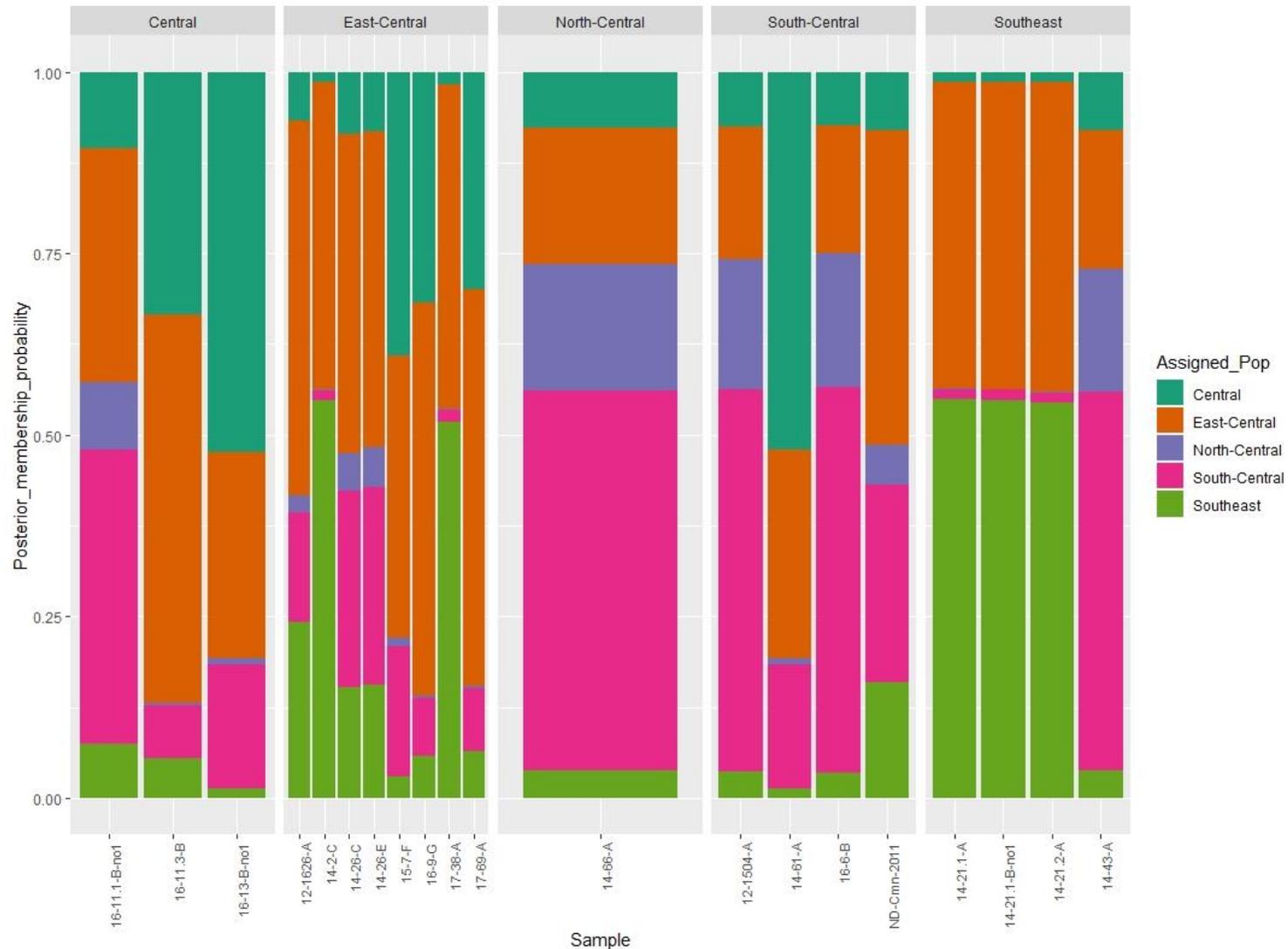
Figure 2.10. Composite stacked bar plot for 20 North Dakota *Clavibacter nebraskensis* isolates using predetermined populations by region (represented by colors in the legend). Each isolate (sample) is represented on the x-axis. The y-axis illustrates the probability of population membership.

Discussion

      This research has identified that aggressiveness differences occur in the ND population of *C. nebraskensis*. Genotypic analyses also indicated genetic differences occur among isolates within the population. However, neither phenotypic nor genotypic variations appear to be related to isolate origin (region or year).

      Three lesion lengths were used to study pathogen infection; ALL, BLL and TLL. *Clavibacter nebraskensis* is documented to be a xylem mobile bacterium and it is likely that infection will progress quickly to the apex of a leaf. Our experiment identified six isolates that were able to develop apical lesions quickly amounting to larger lesion sizes. Similarly, several *C. nebraskensis* isolates had significantly higher BLL, yet the lesion sizes were often smaller than ALL. These results potentially could be used to investigate the affinity for the pathogen to cause the leaf blight or wilt phase of disease development. It was reported that lesion progression has been observed from the leaf apex downwards on seemingly non-wounded plants (Mallowa et al. 2016). Therefore, it is possible that isolates with larger BLL may lead to the wilt phase of Goss's wilt and leaf blight quicker and may help explain the likelihood of seeing the wilt phase of the disease.

      No pattern was observed in the performance (aggressiveness) of isolates from the same field. Three fields were represented by multiple samples in the aggressiveness assay. Isolates collected from the same field displayed both intermediate and most aggressive characteristics. Seven isolates were included from field 16-11, and all but one were determined to be intermediately aggressive. These results indicate that isolates from the same field can differ in aggressiveness. Similarly, the statistical groupings did not reveal a pattern among isolate aggressiveness and isolate origin by either year or geographic location. Differences in the

performance of isolates were not influenced by geography (field location) or time (year collected). These results align with those of Smidt and Vidaver (1987) who found differences in morphology and bacteriophage sensitivity among *C. nebraskensis* isolates, but no relationship between those variables and the origin of the isolate (i.e. from plant tissue or debris, or location within the field).

Differences in *C. nebraskensis* colony characteristics have been previously reported (Ahmad et al. 2015; Smidt and Vidaver 1987). This study documented differences in colony color, morphology, and size. However, no association was observed between any of the colony characteristics and isolates' levels of aggressiveness as all pathogenic isolates were small, mucoid, and orange in color. These characteristics (round, mucoid, 3 to 5 mm in diameter, and apricot-orange in color) are typical of *C. nebraskensis* colonies (Gross and Vidaver 1979). Smidt and Vidaver (1987) saw differences among colony color and morphology of 50 *C. nebraskensis* strains collected from one popcorn field. The 50 strains were separated into four types based on colony color and morphology. Smidt and Vidaver noted variation among their 50 strains but also acknowledged that the sample size likely did not reflect the true level of variation occurring in a natural population. Ahmad et al. (2015) recorded colony morphology of 37 putative *C. nebraskensis* strains and tested their pathogenicity on corn plants inoculated in the greenhouse. Using colony morphology and pathogenicity, they were able to identify 28 of the isolates as *C. nebraskensis*. Thirty-six of the isolates in this study were non-pathogenic, including all those that were large in size, fluidal, and yellow in color.

The linear model chosen to examine lesion proportions from LI-COR area meter measurements and physical measurements at 21 dpi was found to be strong and was able to explain 73% of the variation in the data. This indicates that the area meter may be a valuable tool

for assessing disease severity in future studies. Physical measurements of lesion and leaf lengths and widths throughout the experiment were time-consuming and laborious. Plants had to be moved each time they were measured. Moving plants took time and posed risk for damage to leaves, which could impede lesion development. Also, although lesion and leaf width measurements were taken, there is much natural variability in the width of corn leaves (Daughtry and Hollinger 1984; Sanderson et al. 1981). Due to the variability in lesion width, data were not combinable, and therefore, were not included in statistical analyses. By making one terminal assessment using the LI-COR, much time could be saved during the experiment while still getting accurate results as shown by the correlation analysis. However, processing leaves on the area meter was time consuming as leaves had to be flattened and manually fed onto the conveyor belt, one leaf at a time. Although the area meter could save time during the experiment, a large amount of time needs to be dedicated for measurements at the end of the experiment. Therefore, if only a terminal measurement of lesion length were desired, physical measurements would be appropriate to save time. However, if lesion proportion or percentage severity were the desired terminal measurements, the area meter would be appropriate as it captures all variability in leaf and lesion widths. One drawback to solely making a terminal disease assessment would be that AUDPC could not be calculated and aggressiveness could only be defined by lesion proportion. Physical measurements are therefore necessary to make multiple assessments over the course of an experiment.

Genotypic analyses indicated that although differences are occurring among ND *C. nebraskensis* isolates, these differences are random. Any genetic difference among the isolates had the same chance of occurring in any region or year. Therefore, as there was no clear

differentiation among isolates based on either region or year collected, it was concluded that there is no structure to the current population.

Although not significant, a few observations were made from the results of the genetic analyses. Two fields with multiple pockets of disease were represented within the 20 genotyped isolates. The two isolates from field 14-26 were connected by short branches (less than 5% of loci differed) on the distance tree. Interestingly, 14-26-C grouped as an aggressive isolate, while 14-26-E was moderately aggressive. This could imply that isolates 14-26-C and 14-26-E came from the same source of inoculum. Three isolates from field 14-21 also appeared to be closely related. However, a second short branch (less than 5% of loci differed) separated isolates from pockets within the field, 14-21.1 and 14-21.2. Isolate 14-21.1-B #1 was considered aggressive, while 14-21.2-A was intermediately aggressive and 14-21.1-A failed to cause disease in the aggressiveness assay. Isolates 14-21.1-A and 14-21.1-B #1 could be from the same source of inoculum and isolate 14-21.2-A could be from a second source of inoculum or could be the result of genetic mutation. Two isolates from field 16-11 and one from the nearby field 16-13 were not as closely related (18 to 20% of loci differed). Isolates 16-11.1-B #1, 16-11.3-B, and neighboring isolate 16-13-B #1 possibly could have come from multiple sources of inoculum. Of those three isolates, only 16-11.1-B #1 was considered aggressive. Additional aggressive isolates, 14-43-A, 14-61-A, 14-66-A, 16-6-B, and 16-9-G were dispersed throughout the distance tree.

A study of the *C. michiganensis* subsp. *michiganensis* population in Turkey found that the majority of the population came from a single introduction event (Sen et al. 2018). Similarly, de León et al. (2009) found that the *C. michiganensis* subsp. *michiganensis* population in the Canary Islands came from a single inoculum source. In Argentina, however, it appears that *C. mighiganensis* subsp. *michiganensis* is being introduced every year via seed. In California, the

pathogen has also been introduced multiple times, likely on contaminated seed (Thapa et al. 2017). The use of genetic diversity analyses are useful when monitoring sources of inoculum and pathogen movement.

Aggressiveness and genetic diversity was the focus of study in other bacterial pathosystems. Repetitive-sequence polymerase chain reaction determined genetic diversity within the Uruguayan *Ralstonia solanacearum* population (Siri et al. 2011). Genetic diversity was found to be low among the *R. solanacearum* population, but differences in aggressiveness among isolates was found when the bacterium was inoculated on tomato and potato plants. Pulse-field gel electrophoresis (PFGE) separated *C. michiganensis* subsp. *michiganensi*s stains into 12 groups (Basim and Basim 2018). Although the strains were highly diverse, no correlations existed among pathogenicity, phenotype (described by the percent wilt caused by an isolate), number of plasmids, or genetic diversity. As concluded by the authors, although no correlations were observed, the data is useful for better understanding a population and for monitoring changes in populations in the future.

The current subset of 20 isolates did not encompass all phenotypic differences observed among the 50 isolates included in the aggressiveness assay, and so, association mapping was not appropriate with the current data. The additional 30 isolates should be sequenced in hopes of attaining higher numbers of reads. Although no structure was presently found in the ND *C. nebraskensis* population, in the future, association mapping could be used to determine if any relationship exists between isolate phenotype (aggressiveness) and genotype. Candidate genes for pathogenicity can be identified by evaluating pathogenicity, aggressiveness, and genomic structure of plant pathogens (Lu et al. 2018). Genotyping-by sequencing (GBS) and genome-wide association studies (GWAS) have been utilized in other pathosystems to identify virulence

factors and candidate genes for aggressiveness and mycotoxin production (Gao et al. 2016;

LeBoldus et al. 2015; Muchero et al. 2018; Talas et al. 2016). Such information would be

beneficial to the understanding of *C. nebraskensis* as information is lacking on its pathogenicity,

aggressiveness, and virulence strategies (Agarkova et al. 2011).

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CHAPTER 3. EFFECTS OF HYBRID SUSCEPTIBILITY AND INOCULATION TIMING ON GOSS'S LEAF BLIGHT SEVERITY AND CORN YIELD

Introduction

Goss's wilt and leaf blight, caused by the bacterium *Clavibacter nebraskensis* (Vidaver and Mandel 1974) Li et al. 2018, is a yield-limiting disease of corn. In 2006, the disease re-emerged in the United States Corn Belt after a near 30-year absence (Jackson et al. 2007). The bacterial pathogen has since spread to new regions in the U.S. and Canada, including the state of North Dakota (ND) in 2011 (Friskop et al. 2014). As of 2018, Goss's wilt and leaf blight has been confirmed in Colorado, Illinois, Indiana, Iowa, Kansas, Louisiana, Michigan, Minnesota, Missouri, Nebraska, North Dakota, South Dakota, Texas, Wisconsin, Wyoming, and in Alberta and Manitoba in Canada (Desjardins 2010; Friskop et al. 2014; Howard et al. 2015; Jackson-Ziems et al. 2012; Korus et al. 2011; Malvick et al. 2010; Ruhl et al. 2009; Singh et al. 2015; Sweets and Hosack 2014). Goss's wilt and leaf blight was considered a top-ten disease in the northern corn growing region of the U.S. and Ontario, Canada from 2012 to 2015, and is considered the most important corn disease in ND (Friskop and Bauske 2017; Mueller et al. 2016). National yield losses caused by Goss's wilt and leaf blight were estimated to be in excess of 12.7 billion kg over a four-year period (Mueller et al. 2016).

Yield loss attributed to Goss's wilt and leaf blight has been assessed using both observational data and field research data. Under high levels of disease, yield losses as high as 3,700 kg/ha were reported in northwest Indiana (Wise et al. 2010), losses of 30% have been reported in Minnesota (Malvick 2018), and the use of very susceptible hybrids resulted in yield losses exceeding 50% (Claflin 1999). Inoculated field trials reported yield losses of 55% on susceptible hybrids (Malvick et al. 2014) and losses as high as 44% on susceptible inbred lines

(Carson and Wicks 1991). Although these reports have documented the importance of the disease on susceptible hybrids and inbred lines, the extent of yield loss will vary depending on the level of host resistance and timing of disease onset. Calub et al. (1974b) indicated inoculation timings completed on two-week-old seedlings routinely resulted in higher disease severity than inoculations on four-, six-, or eight-week-old seedlings. Resistant crosses had significantly less disease when inoculated after eight weeks of growth than did susceptible material. Additionally, disease ratings on resistant material decreased as age at inoculation increased. Inoculation timings on susceptible sweet corn indicated disease severity was highest and yield was lowest when inoculated at the three-to-five leaf stage (Suparyono and Pataky 1989a). However, when a resistant hybrid was used, inoculation timing had very little impact on either disease severity or yield.

Management of Goss's wilt and leaf blight is best accomplished using an integrated approach of residue management, crop rotation, and host resistance. Incorporating corn residue into the soil surface has been shown to significantly reduce disease levels (Mehl et al. 2014). The bacterium survives on host residue for approximately 10 months and an extended rotation away from corn can reduce primary inoculum (Schuster 1975; Smidt and Vidaver 1986). Host resistance is often the preferred tool for managing this disease. Genetic resistance to Goss's wilt and leaf blight is quantitative and several significant quantitative trait loci (QTLs) have been identified (Cooper et al. 2018; Singh et al. 2016; Treat and Tracy 1990). However, no hybrids are immune to *C. nebraskensis* (Pataky 1985). The use of resistant and moderately resistant hybrids can significantly reduce disease severity and prevent substantial yield loss (Carson and Wicks 1991; Malvick et al. 2014; Pataky et al. 1988). Another management tool that has been explored is the use of chemical treatments. Plant protection products, such as copper and hydrogen

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peroxide, have been tested and are not considered a viable management option for Goss's wilt and leaf blight (Korus et al. 2010; Mehl et al. 2015).

Corn grown in ND ranges from 75 to 102 relative maturity (RM). The length of the growing season impacts the corn plant's ability to increase dry matter for yield (Ransom et al. 2004). Stress to the plant at the silk stage (R1) has the largest impact on yield (Ransom 2013). Deficient pollination and seed set can be the result of moisture stress, such as that caused by *C. nebraskensis*, at R1. In ND, *C. nebraskensis* infection can occur during early vegetative leaf stages or after tasseling. The leaf blight phase of the disease is most common in ND, with the wilt phase being infrequently documented. Therefore, Goss's wilt and leaf blight is hereafter referred to as Goss's leaf blight. Documenting the yield loss associated with disease onset on hybrids with varying levels of resistant is crucial when developing management recommendations for northern corn production. Therefore, the objective of this study was to evaluate the effects of hybrid susceptibility and inoculation timing on Goss's leaf blight severity and yield.

Materials and Methods

Research sites, hybrids, and inoculations timings

Between 2015 and 2017, six field experiments were conducted in four locations in ND. In 2015, trials were conducted on grower-cooperators' land near Harwood (HAR) and Hazelton (HAZ). In 2016 and 2017, research sites were established at the North Dakota State University Agronomy Seed Farm near Casselton (CASS) and in a cooperator's field near Kindred (KIND). Three Dekalb hybrids (DKC37-38RIB, DKC36-30RIB, and DKC33-78RIB) were selected based on ratings for resistance to Goss's wilt and leaf blight. The rating scale used by Dekalb extends from 1 to 9, with 1 to 2 = excellent, 3 to 4 = very good, 5 to 6 = good, 7 to 8 = fair, and 9 = poor

(Monsanto Co., St. Louis, MO). Hybrid DKC37-38RIB has a RM of 87 days and a Goss's wilt rating of 7 (susceptible); DKC36-30RIB has a RM of 86 days and a Goss's wilt rating of 5 (moderately susceptible); and hybrid DKC33-78RIB has a RM of 83 days and a Goss's wilt rating of 4 (resistant). Inoculation timings consisted of a non-inoculated treatment, an early-season inoculation when six to ten leaves were present (V6 to V10), a late-season inoculation at the reproductive silk stage (R1), and a treatment that received both an early-season (V6 to V10) and a late-season inoculation (R1).

Experimental design

The trial design was a randomized complete block arranged as a split-plot with four replications. Corn hybrid was the whole plot factor, while inoculation timing served as the sub-plot factor. The hybrid blocks were randomized within each replicate and the inoculation timings were randomized within each hybrid block. The same three hybrids were used across all research sites. Three inoculation timings were used in 2015 (non-inoculated, V6 to V10, and R1), while four inoculation timings (non-inoculated, V6 to V10, R1, and the combination of V6 to V10 and R1) were used in 2016 and 2017. Four-row plots were established with the center two rows receiving the inoculation treatment. In 2015, plots were planted 9.1 m in length and were reduced to 7.3 m. In HAZ, plots were planted with a John Deere 1770NT 16-row vacuum planter (John Deere, Moline, IL). Row spacing was 76.2 cm and seed spacing was 16.5 cm. In HAR, CASS, and KIND, a Monosem Runabout two-row vacuum planter was used (Monosem, Hutchinson, KS). Seed spacing was 15.2 cm. Due to smaller land allocations in 2016 and 2017, plots were planted 7.6 m in length and reduced to 6.1 m. Plot rows one and four were planted to the resistant hybrid, DKC33-78, to prevent dissemination of the pathogen between treatment plots following inoculations. All trial agronomics are presented in Table 3.1.

Table 3.1. Agronomic details and disease pressure information for hybrid by inoculation timing trials conducted in 2015 to 2017.

Location	Planting date	Row width (cm)	Plot length (m)	Seed spacing (cm)	Early inoculation		Late inoculation		Harvest date	Disease pressure	Mean yield (kg/ha)[y]	Mean tw (kg/hL)[z]
					Date	Growth stage	Date	Growth stage				
HAZ15	May 5	76.2	7.3	22.1	July 14	V7-V10	August 5	R1	October 1	Low	-	-
HAR15	May 5	76.2	7.3	15.2	July 15	V6-V8	August 7	R1	October 8	Low	-	-
CASS16	May 12	76.2	6.1	15.2	June 29	V8	July 26	R1	October 10	Low	14,220	76.0
KIND16	June 2	76.2	6.1	15.2	July 5	V6	August 1	R1	October 21	High	11,929	74.5
CASS17	May 19	76.2	6.1	15.2	July 6	V8-V9	August 1	R1	October 20	High	11,921	71.7
KIND17	June 2	76.2	6.1	15.2	July 5	V8	July 31	R1	October 25	High	13,778	74.0

[y]Represents the mean yield in kilograms per hectare of non-inoculated control plots.
[z]Represents the mean test weight in kilograms per hectoliter of non-inoculated control plots.

Inocula production and inoculation procedure

In 2015, the *C. nebraskensis* isolate used to inoculate both trial locations was 'ND Cmn 2011', the original *C. nebraskensis* isolate found in Emmons County in 2011 (Friskop et al. 2014). In 2016 and 2017, *C. nebraskensis* isolate 15-28-A (isolate collected from Emmons County, ND in 2015) was used. In 2015, bacterial cultures were grown on nutrient broth yeast (NBY) media for 96 h, scraped, centrifuged, and then suspended in 10 mM potassium phosphate buffer (PB) to a concentration of 10^6 CFU/ml. The bacteria were centrifuged for 22 min at 4,000 x g. After centrifugation, the supernatant was poured off and bacterial pellets were resuspended in PB. Inoculum concentration was tested on a NanoDrop 1000 Spectrophotometer at a wavelength of 600 nm. An optical density of approximately 0.20 represented the target concentration of 10^6 CFU/ml.

For the early-season inoculations in 2016, three-day-old cultures of *C. nebraskensis* 15-28-A grown on NBY were scraped with 3 ml of PB for a targeted concentration of 1×10^8 CFU/ml. Inoculum concentration was increased from 1×10^6 CFU/ml to 1×10^8 CFU/ml in hopes of creating higher disease pressure than experienced in 2015. For the late-season inoculations, freshly ground infected tissue was also added to prepared *C. nebraskensis* inocula. Approximately two to three leaves were added per one liter of inoculum buffer. Due to dry weather at the CASS location, an additional inoculation occurred two weeks after the late-season inoculation. Plots were inoculated using a bacterial suspension of freshly ground leaves (approximately two to three infected leaves per liter of PB) and a STIHL SR 450 backpack sprayer/duster (STIHL Inc., Virginia Beach, VA).

In 2017, ground infected leaf tissue was the sole source of *C. nebraskensis* inocula. Alterations to the protocol described by Mehl et al. (2015) were made to produce *C.*

nebraskensis inocula. Potassium phosphate was used as the buffer instead of NaCl and a ratio of 5 infected leaves per 500 ml of buffer was used in place of 10 leaves per 3.8 liters 0.1 M NaCl. A Ninja Professional Blender, model BL610 (Euro-Pro Operating LLC, Newton, MA) was used for grinding leaf tissue. Due to the volume restriction of the Ninja pitcher (1900 ml), batches of 1000 to 1500 ml were blended for 30 s at a time.

A modified pin-prick method was used for mechanical inoculations (Calub et al. 1974a). Inoculating tools consisted of stainless steel grill tongs outfitted with a sponge and a rubber pad with nails (Figure 3). The nails created a wound and entry point for the bacteria on corn leaves, while the sponge held the inoculum. At the early-season inoculation, inoculating tools were clamped onto three to four of the upper-most leaves on every plant in each treatment row. At the late-season inoculation, the ear leaf and the uppermost fully-extended leaf were inoculated. In 2016 and 2017, to help create conducive conditions (humidity) for disease development, treatment rows were misted with water using a STIHL SR 450 backpack sprayer a few minutes prior to inoculation. Inoculations were conducted late in the afternoon to limit ultraviolet light damage to the pathogen. This provided longer periods of leaf wetness and humidity in the canopy of treatment rows.
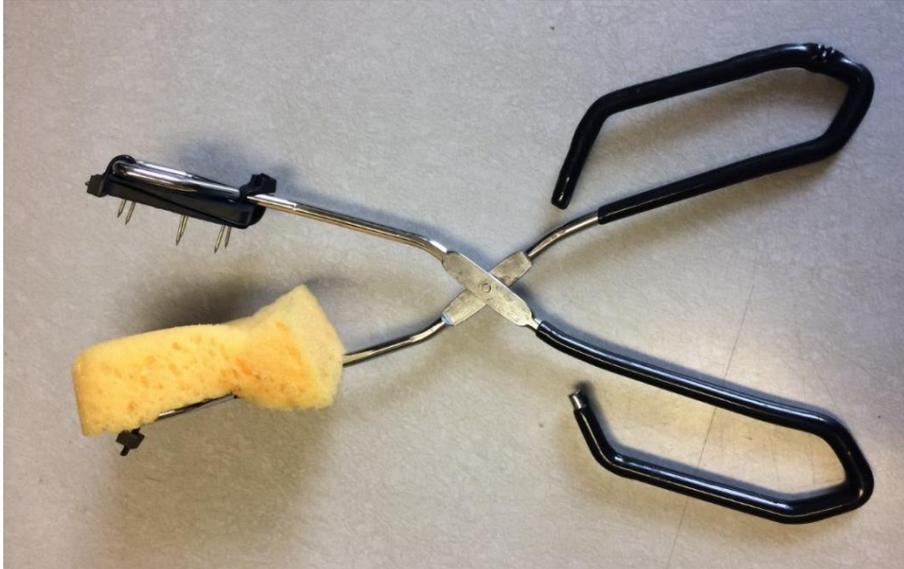
Figure 3.1. Inoculating tool for *Clavibacter nebraskensis* inoculations. Tool is fitted with nails to create a wound and a sponge is used to absorb inocula for insertion into corn leaves.

Disease assessment and data collection

Beginning two to four weeks following the early-season inoculation, each plant in the treatment rows was evaluated for disease incidence, and mean disease severity was obtained from at least 10 arbitrarily selected plants per plot. Disease incidence was calculated by dividing the number of infected plants by the total amount of plants in each plot. Disease severity was evaluated using a 0 to 100% leaf severity scale and a mean percentage severity was generated for each evaluated plant. In 2017, due to high levels of disease, lower and upper canopy severity ratings were recorded, and mean canopy ratings were used for data analyses. Disease severity data were used to calculate area under the disease progress curve (AUDPC) as follows (Shaner and Finney 1977):

$$\sum_{i=1}^{n}[(Y_{i+1} + Y_{i})/2]\,[X_{i+1} - X_{i}]$$

where Y_i = percentage disease severity at the i*th* observation and X_i = time in days at the i*th* observation. To help standardize disease epidemics at each location, relative area under the

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disease progress curve (RAUDPC) was calculated by dividing final AUDPC by the length of time between the first and last disease evaluations in a season. Plots were either hand-harvested or combined using a Zürn 150 universal plot harvester (Zürn Harvesting GmbH & Co. GK, Ravenstein, Germany). Corn ears from hand-harvested plots were shelled using either the Zürn 150 universal plot harvester or for KIND17, an ALMACO ECS Bulk Ear Corn Sheller (ALMACO, Nevada, IA). Yield parameters were obtained with a High Capacity GrainGage and Mirus Harvest Software (Juniper Systems & HarvestMaster, Logan, UT) and yield was calculated at 15.5% moisture. For KIND17, test weight and moisture were measured with a DICKEY-john GAC500XT grain moisture tester (Auburn, IL). Yield and test weight loss were calculated for each inoculated plot by subtracting its yield and test weight from the corresponding non-inoculated plot within the same hybrid and replication. Percentage yield loss and test weight loss were calculated by dividing each loss value by the yield or test weight of the corresponding non-inoculated plot, then multiplying by 100.

Data analysis

Analysis of variance (ANOVA) was used on AUDPC data for hybrid and inoculation timing from each location separately in Statistical Analysis Software (SAS) (v. 9.4; SAS Institute Inc., Cary, NC). Individual analyses for severity data and yield data from sites with successful inoculation events (KIND16, CASS17, and KIND17) showed similar trends across sites therefore a combined analysis was used to best represent the data. Using the general linearized mixed model (GLIMMIX) in SAS, the RAUDPC and yield loss data was analyzed for hybrid, inoculation timings and subsequent interactions. Environment and replication were considered random effects and hybrid and inoculation timing were considered fixed effects. Significant differences in least squares means (LS Means) data were evaluated using Fisher's least

significant difference (LSD) test at $\alpha = 0.05$. Significant linear relationships between R1 disease severity and yield were analyzed for each hybrid at the 95% level of confidence using PROC CORR in SAS and Pearson correlation coefficients were used to observe strengths of relationships.

Results

Research site disease levels

Extenuating circumstances in 2015 resulted in no data being obtained from either trial location. At the HAR location, white-tailed deer fed on three of the four replications while the crop was in vegetative growth stages. The growing points were damaged on most plants, thus no ears developed. At the HAZ location, natural infection by *C. nebraskensis* set in prior to the early-season inoculation and confounded inoculation-timing results. In 2016, inoculations at the KIND location were successful. At 10 days post-inoculation (dpi), symptom development was documented and a high level of disease was apparent at season's end. Only one successful inoculation event at R1 occurred prompting disease incidence levels at CASS to be very low. In 2017, disease incidence was high at both KIND and CASS.

Disease assessment

Significant interactions of hybrid by inoculation timing were present. However, the interaction was due to differences in magnitude and AUDPC values for hybrid and inoculation timing are presented separately for KIND16, KIND17, and CASS17 (Figures 3.2 and 3.3). Significant differences existed among AUDPC values for both hybrid and inoculation timing. At each location, differences in disease progression among hybrids were present by the first evaluation date. At all locations and assessment dates, disease progression on the susceptible hybrid was significantly greater than that on the moderately susceptible or resistant hybrids. No

significant differences in disease progression occurred between the V6 to V10 and V6 to V10 and R1 inoculations. Beginning at the first assessment date, disease progression in plots receiving the V6 to V10 or V6 to V10 and R1 inoculations was significantly greater than the non-inoculated or R1-inoculated plots. At KIND17 and CASS17, disease progression in R1-inoculated plots surpassed that of non-inoculated plots at the final and second-to-last assessment date, respectively.
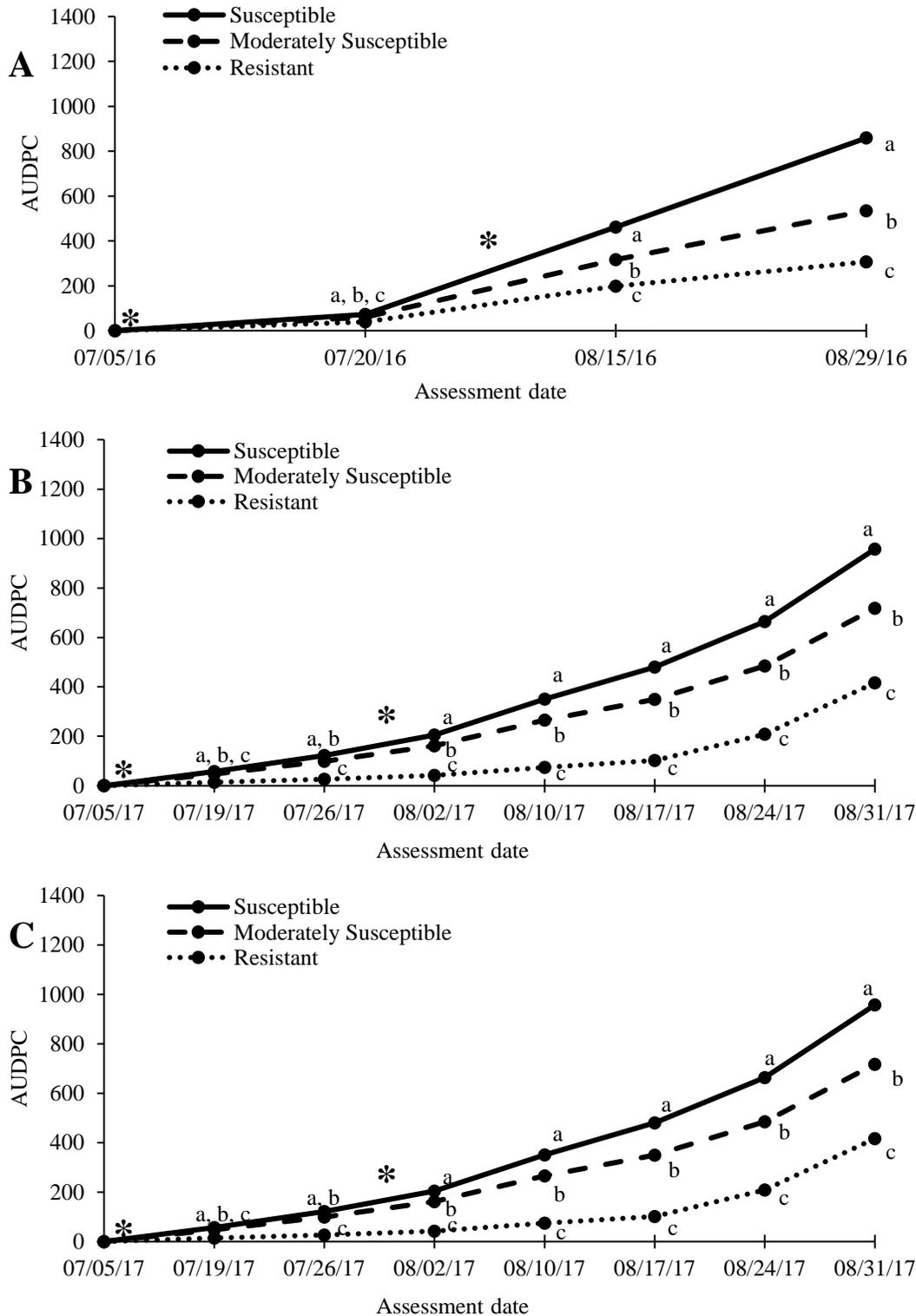
Figure 3.2. Area under the disease progress curves (AUDPC) for hybrids at (A) KIND16, (B) KIND17, and (C) CASS17. Inoculation events are represented by (*). AUDPC values followed by the same letter are not significantly different based on Fisher's least significant difference (α = 0.05).
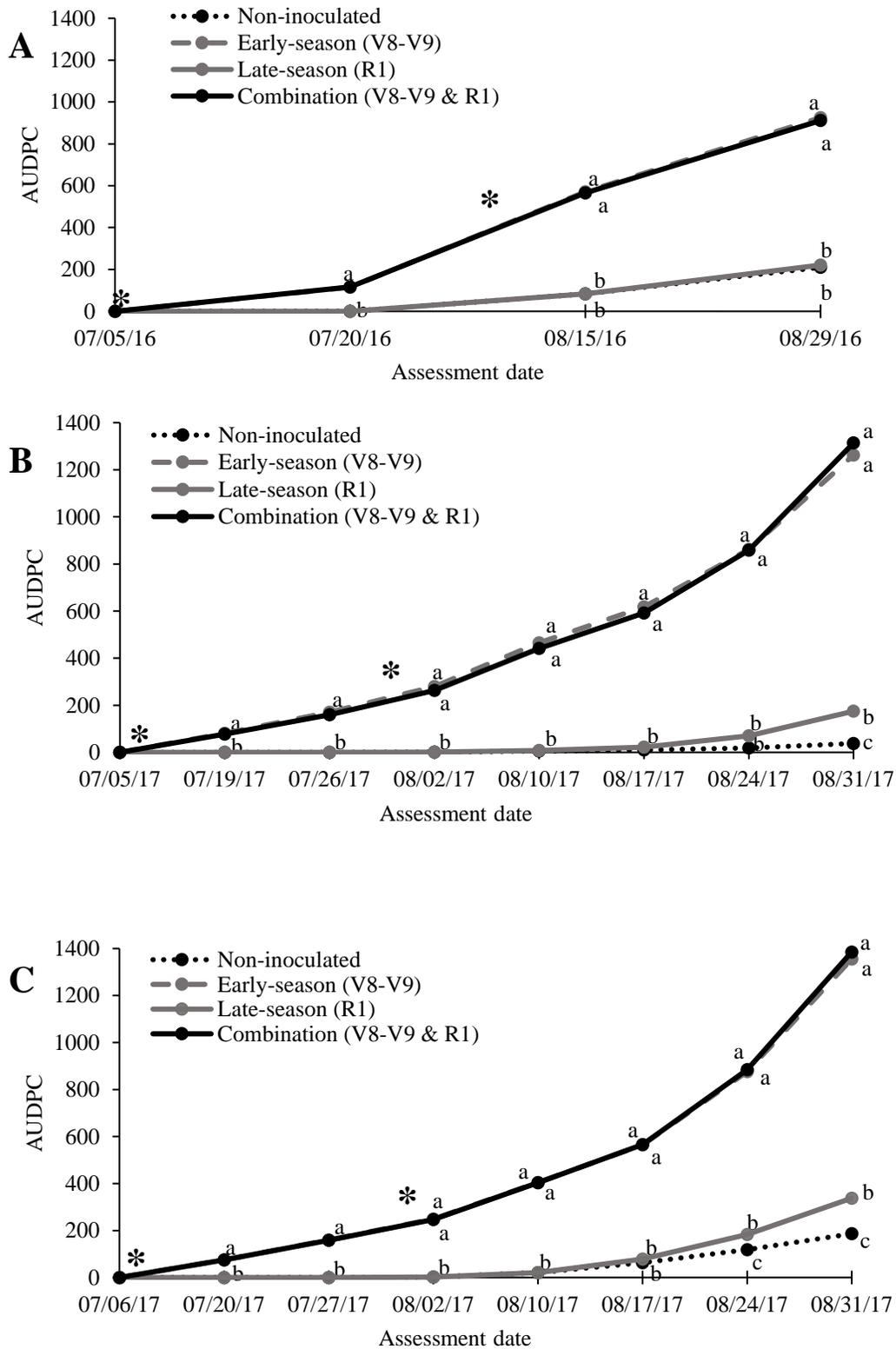
Figure 3.3. Area under the disease progress curves (AUDPC) for inoculation timings at (A) KIND16, (B) KIND17, and (C) CASS17. Inoculation events are represented by (*). AUDPC values followed by the same letter are not significantly different based on Fisher's least significant difference (α = 0.05).

The combined analysis of RAUDPC and R1 data from KIND16, KIND17, and CASS17 indicated a significant interaction between hybrid and inoculation timing. The interactions for both dependent variables were due to magnitude, thus the effects of hybrid and inoculation timing are presented separately (Table 3.2). Significant differences in disease severity were observed among hybrids at R1. The susceptible hybrid had the highest disease severity followed by the moderately susceptible hybrid then the resistant hybrid. Similarly, the RAUDPC values for the susceptible hybrid were significantly higher than the moderately susceptible and resistant hybrids. Significant differences in R1 disease severity and RAUDPC were also observed among inoculation timings. Disease severity at R1 was statistically highest for both inoculation treatments that included an early-season inoculation event. Disease was observed in the non-inoculated treatment (due to late-season pathogen spread into plots), yet R1 severity values were statistically lower than the other inoculation treatments. The LS Means RAUDPC values for inoculation treatments including an early-season inoculation were statistically similar to each other, yet statistically higher than the non-inoculated and R1 inoculation event.

Table 3.2. Combined analysis results (KIND16, KIND17 and CASS17) for disease severity (percentage) at silking (R1) and relative area under the disease progress curve (RAUDPC) for hybrid and inoculation timing.

	R1 disease severity (%)	RAUDPC
Hybrid		
Susceptible	21 a^z	0.18 a
Moderately susceptible	12 b	0.12 b
Resistant	5 c	0.07 c
Timing		
Non-inoculated	5 c	0.03 b
Early-season	19 a	0.21 a
Late-season	7 b	0.04 b
Combination	19 a	0.21 a

^{z}LS Means followed by the same letter are not significantly different based on Fisher's least significant difference ($\alpha = 0.05$).

For CASS16, the early-season inoculation was not successful, and only hybrids were analyzed using data from late-season inoculation events. At season-end, both the susceptible and moderately susceptible hybrids had significantly higher disease severity than the resistant hybrid (Table 3.3).

Table 3.3. Season-end disease severity (percentage) and yield loss (kg/ha) for CASS16. Data are presented for late-season (R1) and combination (V8 and R1) inoculation treatments only. Values in parentheses following yield loss indicate the percentage yield loss.

Hybrid	Final disease severity (%)	Yield loss[z] (kg/ha)
Susceptible	10 a[y]	199 (1)
Moderately susceptible	7 a	794 (5)
Resistant	2 b	-57 (-1)
P-value[x]	0.0047	0.3740 (0.3937)
LSD	4	NS

[x]Level of significance (*P*-value) for analysis of variance at the 95% level of confidence using the general linearized mixed model.
[y]Means followed by the same letter are not significantly different based on Fisher's least significant difference ($\alpha = 0.05$).
[z]Yield loss is the difference in yield of inoculated plots from corresponding non-inoculated plots.

Yield assessment

A significant hybrid by inoculation timing interaction was present for yield parameters. Therefore, yield parameters are reported for each hybrid individually. Significant differences in yield loss were observed among inoculation timings (Table 3.4). Whether stand-alone or in combination with the R1 inoculation, the V6 to V10 inoculation resulted in significantly greater yield loss than the single R1 inoculation in both the susceptible and moderately susceptible hybrids (Table 3.4). Yield losses of 34 to 41% and 22 to 25% were documented for V6 to V10 inoculation events in the susceptible and moderately susceptible hybrids, respectively. No statistical differences in yield loss were observed among inoculation timings in the resistant hybrid, with yield loss ranging from 3 to 11% across timings (Table 3.4). While not statistically comparable, the R1 inoculation resulted in numerically greater yield loss in the moderately susceptible hybrid than it did in the susceptible or resistant hybrids. Numerically, hybrids

followed the same trend with the single R1 inoculation resulting in the lowest yield loss and the combination V6 to V10 and R1 inoculations resulting in the greatest numerical yield loss.

Table 3.4. Analysis for yield loss represented in kg/ha and as a percentage for hybrids at each inoculation timing.

Hybrid	Timing	Yield Loss[y] (kg/ha)	Yield Loss (%)
Susceptible	V6 to V10	3,889 a[z]	34 a
	R1	469 b	2 b
	V6 to V10 and R1	4,621 a	41 a
	LSD	1,467	14
Moderately susceptible	V6 to V10	3,039 a	22 a
	R1	1,186 b	8 b
	V6 to V10 and R1	3,427 a	25 a
	LSD	1,459	14
Resistant	V6 to V10	853 NS	6 NS
	R1	522 NS	3 NS
	V6 to V10 and R1	1,474 NS	11 NS
	LSD	1,459	14

[y]Yield loss is the difference in yield of inoculated plots from their corresponding non-inoculated plots.
[z]LS Means followed by the same letter are not significantly different based on Fisher's least significant difference ($\alpha = 0.05$).

No significant differences in yield parameters were present at CASS16. Yield loss ranged from -57 kg/ha to 199 kg/ha in the resistant and susceptible hybrids, respectively. Numeric yield losses corresponded to yield loss percentages of 1%, 5%, and -1% in the susceptible, moderately susceptible, and resistant hybrids, respectively (Table 3.3).

Significant differences in test weight loss and percentage loss were present for the main effect of hybrid (Table 3.5). Test weight losses ranged from 0.1 kg/hL to 1.3 kg/hL on the resistant and susceptible hybrids, respectively. The corresponding test weight loss percentages were 0.1% and 1.8% on the resistant and susceptible hybrids, respectively (Table 3.5). Furthermore, no significant differences existed for test weight loss among hybrids ($P = 0.3719$) or inoculation timings ($P = 0.6285$) for CASS16 (data not shown).

Table 3.5. Combined test weight loss represented as kg/hL and as a percentage for hybrids across inoculation timings.

Hybrid	Test weight loss[y] (kg/hL)	Test weight loss (%)
Susceptible	1.3 a[z]	1.8 a
Moderately susceptible	0.7 ab	1.0 ab
Resistant	0.1 b	0.1 b
P-value[x]	0.0295	0.0228

[x]Level of significance (*P*-value) for analysis of variance at the 95% level of confidence using the general linearized mixed model.

[y]Test weight loss is the difference in test weight of inoculated plots from their corresponding non-inoculated plots.

[z]Means followed by the same letter are not significantly different based on Fisher's least significant difference ($\alpha = 0.05$).

Correlation

Correlations were determined between R1 disease severity and yield (kg/ha) for the susceptible, moderately susceptible, and resistant hybrids (Figure 3.4). Significant linear relationships existed between R1 disease severity and yield for each respective hybrid ($P = <0.0001$, 0.0376, and 0.0124 for the susceptible, moderately susceptible and resistant hybrids, respectively) (Figure 3.3). The Pearson correlation coefficients for R1 disease severity and yield were -0.59, -0.30, and -0.36 for the susceptible, moderately susceptible, and resistant hybrids, respectively.
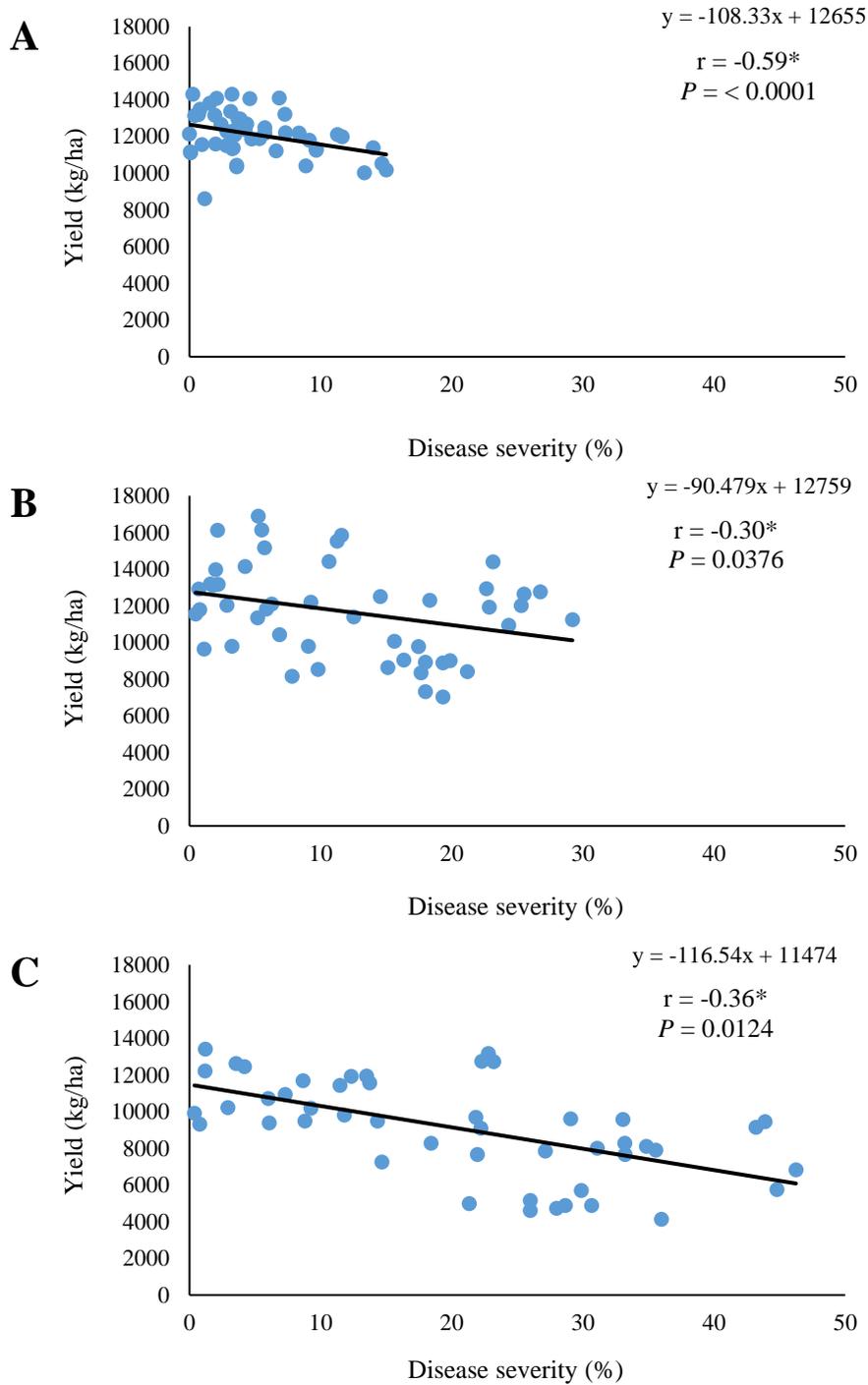
Figure 3.4. Relationships between R1 disease severity (percentage) and yield (kg/ha) for each hybrid: (A) susceptible, (B) moderately susceptible, and (C) resistant. Pearson correlation coefficients followed by (*) indicate a significant linear relationship between variables (α = 0.05).

Discussion

This study documents the impacts of host resistance and inoculation timing on yield loss associated with Goss's leaf blight in northern corn hybrids. Across field experiments, a high level of disease developed in three trials and a low level of disease developed in one trial. The varying levels of disease were able to document significant yield loss on susceptible and moderately susceptible hybrids and quantified the yield loss associated with early- and late-season infection events. Given that corn grown in ND has relatively few diseases and Goss's wilt and leaf blight is the most important corn disease in the state, it is prudent for growers in ND to select hybrids with adequate Goss's wilt and leaf blight resistance.

Previous work on yield loss has focused on inbred lines of corn (Carson and Wicks 1991) and sweet corn hybrids (Suparyono and Pataky 1989a). The results from this study follow a similar trend in what was reported in those studies. Maximum mean yield loss percentages for this study were 41%, 25%, and 11% for the susceptible, moderately susceptible, and resistant hybrids respectively. Carson and Wicks (1991) reported mean yield losses of 40.3% and -0.8% for susceptible and resistant inbred lines, respectively. Similarly, Suparyono and Pataky (1989a) reported marketable ear losses up to 95%, 39%, and 32% on susceptible, moderately susceptible, and moderately resistant sweet corn hybrids, respectively, when inoculations occurred at the three-to-five-leaf stage. Regardless of hybrid susceptibility, results from this experiment indicated that late-season infection events resulted in significantly lower yield loss than early-season infection events. Interestingly, the level of yield loss associated with the late-season inoculation was numerically greatest on the moderately susceptible hybrid. This suggests that DKC36-30 may be more sensitive to late-season infection events and further research on the effect of late-season infection events on several hybrids is needed.

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In the susceptible hybrid, yield was reduced 117 kg/ha for every 1% increase in disease severity at R1. Yield was reduced 90 kg/ha and 108 kg/ha for every 1% increase in disease severity at R1 in the moderately susceptible and resistant hybrids, respectively. Although the linear relationships between R1 disease severity and yield were significant for all three hybrids, only the susceptible hybrid (r = -0.59) appears to have a strong correlation between the two variables. Across locations, R1 severity values of almost 50% were documented in the susceptible hybrid, while maximum severities at R1 were approximately 30% and 15% for the moderately susceptible and resistant hybrids, respectively. The high levels of infection and yield loss that are possible on the susceptible hybrid may be an explanation for the strong correlation between R1 severity and yield. On the other hand, as previously discussed, the moderately susceptible hybrid had a greater numeric yield reduction to infection at R1 than to infection occurring at vegetative stages. This could explain its low correlation (-0.30) to R1 severity and yield. The resistant hybrid also had a low correlation (-0.36) between R1 severity and yield, which is likely due to the overall insignificant reduction in yield observed for the hybrid across locations. In support of our findings, Suparyono and Pataky (1989b) reported a significant linear relationship between sweet corn yield and Goss's wilt incidence. Yield (in terms of ear weight and total number of marketable ears) was reduced approximately 1.5% for each 10% increase in disease incidence from inoculations at the five-to-seven-leaf stage. Significant linear relationships did not exist between yield and disease incidence on the moderately resistant or resistant sweet corn hybrids inoculated at the same growth stage (Suparyono and Pataky 1989b).

It was previously reported that the impact of bacterial wilt (i.e. Goss's wilt and leaf blight) on yield was influenced by the level of host resistance as well as plant age at the time of infection (Suparyono and Pataky 1989a). High correlations were also reported by Carson and

Wicks (1991) in their study of yield loss in 42 inbred lines inoculated with *C. nebraskensis*. Plants were inoculated twice, at the V4 to V6 and V8 to V10 growth stages, and correlation coefficients between disease ratings and percentage yield loss were 0.65 and 0.63 for year one and year two of their study, respectively. Therefore, these findings are all in support of the idea that yield loss is impacted by the timing of infection and susceptibility of a hybrid to Goss's wilt and leaf blight.

Five corn maturity zones exist in ND including zone 1 (92 to 102 RM), zone 2 (90 to 95 RM), zone 3 (85 to 90 RM), zone 4 (75 to 85 RM), and zone 5 ($\leq$ 75 RM) (Ransom et al. 2004). This study used hybrids from one company that varied in host resistance and belonged to corn maturity zone 3. Host resistance is available for all five corn maturity zones in ND, however it is unclear whether yield loss to Goss's wilt and leaf blight would be influenced by RM. Yield loss reports from other studies are often presented with no indication of RM. Follow-up studies investigating potential yield losses in susceptible hybrids of different RM will help strengthen our understanding of Goss's wilt and leaf blight yield loss in ND.

Corn disease surveys in ND often find Goss's wilt and leaf blight in pockets sporadically occurring throughout a field. Also, these pockets are commonly noticed after tasseling and into early reproductive stages of corn development. The results of this study successfully address several questions that are posed by agricultural professionals on yield loss associated with early- and late-season infection events by the Goss's wilt and leaf blight pathogen. To help illustrate the usefulness of this data, a hypothetical corn field with a yield potential of 200 bu/A (12,554 kg/ha) will be used. If a susceptible hybrid is grown and widespread infection occurs early in the season (V6 to V10), yield losses of up to 40 bu/A (2505 kg/ha) could occur. If corn prices range from $3.50 to $4.50, a grower may face an economical loss of $140 to $180 per acre. If the same

grower planted a resistant hybrid (same yield potential), an early-season infection could result in a loss of 19 bu/A (1163 kg/ha) or $66.50 to $85.50 per acre. By planting a resistant hybrid instead of a susceptible hybrid, the grower could potentially save $73.50 to $94.50 per acre. The more difficult question to quantify is the yield loss observed in a field with a sporadic occurrence of Goss's wilt and leaf blight late in the season (difficult to assess yield loss in a field with aggregated pockets). However, it can be concluded that yield loss is still occurring from a late-season infection event, and more importantly, diagnosis of the disease late in the growing season will re-emphasize the importance of using resistant hybrids to avoid deleterious yield losses in subsequent years. This example highlights benefits of genetic resistance in mitigating loss associated with Goss's wilt and leaf blight in ND.

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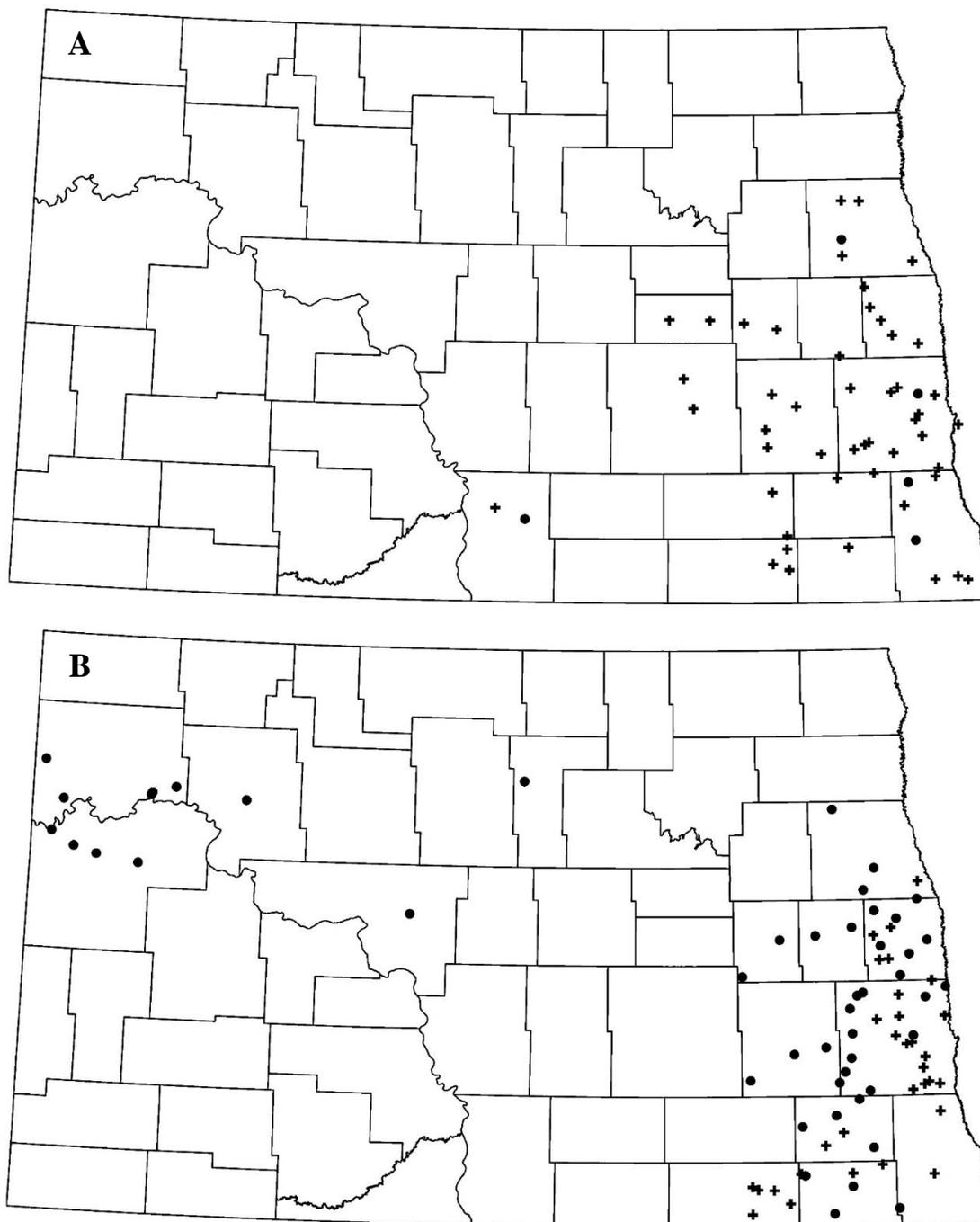
Figure A.1. Distribution of surveyed fields during the 2014 (A), 2015 (B), 2016 (C), 2017 (D), and 2018 (E) foliar disease survey of North Dakota corn fields. Locations designated as (●) represent fields where common rust was not identified, while locations designated as (+) represent fields in which common rust was present.

Figure A.1. Distribution of surveyed fields during the 2014 (A), 2015 (B), 2016 (C), 2017 (D), and 2018 (E) foliar disease survey of North Dakota corn fields. Locations designated as (•) represent fields where common rust was not identified, while locations designated as (+) represent fields in which common rust was present (continued).

Figure A.1. Distribution of surveyed fields during the 2014 (A), 2015 (B), 2016 (C), 2017 (D), and 2018 (E) foliar disease survey of North Dakota corn fields. Locations designated as (•) represent fields where common rust was not identified, while locations designated as (+) represent fields in which common rust was present (continued).
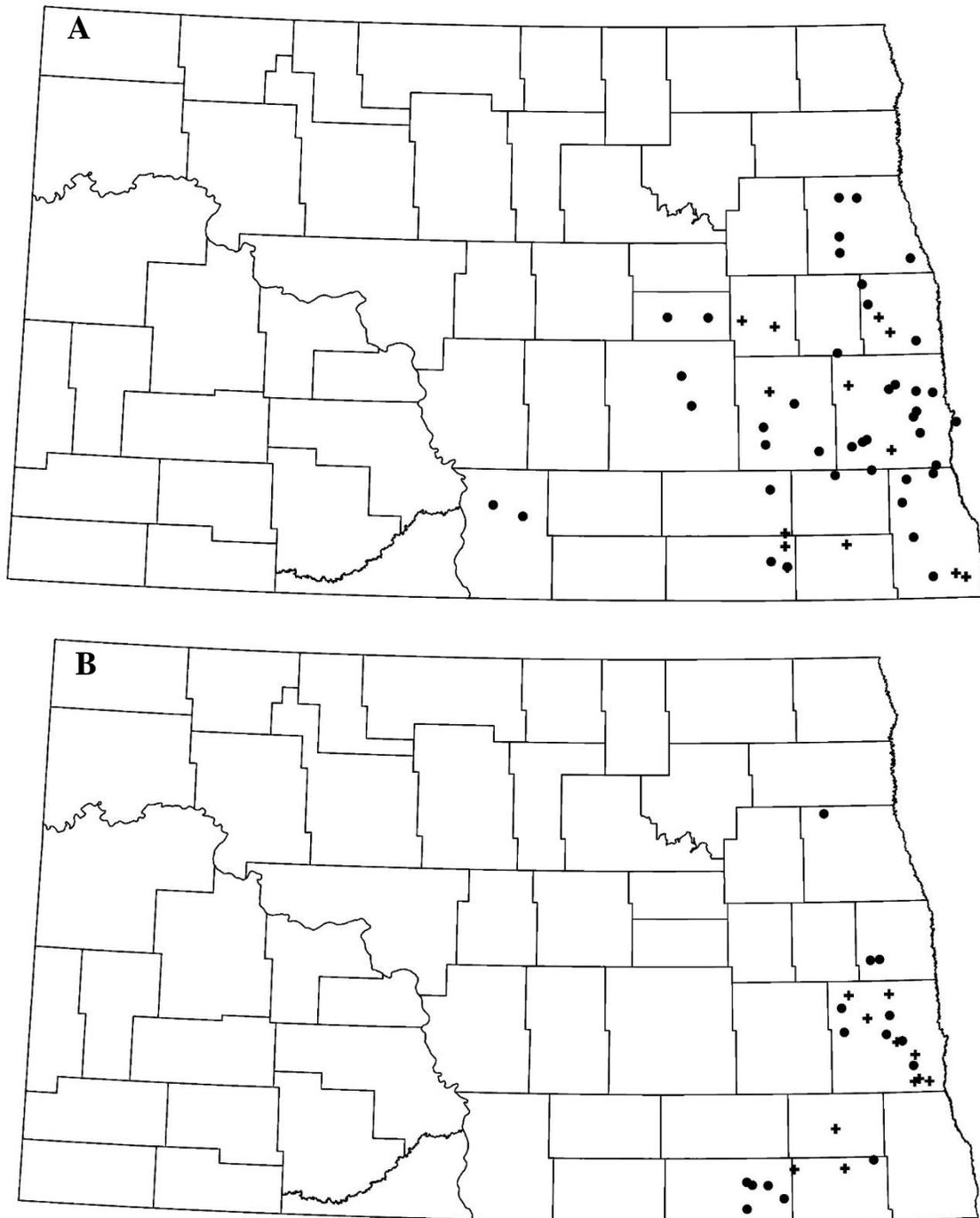
Figure A.2. Distribution of surveyed fields during the 2014 (A), 2015 (B), 2016 (C), 2017 (D), and 2018 (E) foliar disease survey of North Dakota corn fields. Locations designated as (●) represent fields where common smut was not identified, while locations designated as (+) represent fields in which common smut was present.

Figure A.2. Distribution of surveyed fields during the 2014 (A), 2015 (B), 2016 (C), 2017 (D), and 2018 (E) foliar disease survey of North Dakota corn fields. Locations designated as (●) represent fields where common smut was not identified, while locations designated as (+) represent fields in which common smut was present (continued).

Figure A.2. Distribution of surveyed fields during the 2014 (A), 2015 (B), 2016 (C), 2017 (D), and 2018 (E) foliar disease survey of North Dakota corn fields. Locations designated as (●) represent fields where common smut was not identified, while locations designated as (+) represent fields in which common smut was present (continued).

Figure A.3. Distribution of surveyed fields during the 2014 (A), 2015 (B), 2016 (C), 2017 (D), and 2018 (E) foliar disease survey of North Dakota corn fields. Locations designated as (●) represent fields where northern corn leaf blight was not identified, while locations designated as (+) represent fields in which northern corn leaf blight was present.

Figure A.3. Distribution of surveyed fields during the 2014 (A), 2015 (B), 2016 (C), 2017 (D), and 2018 (E) foliar disease survey of North Dakota corn fields. Locations designated as (●) represent fields where northern corn leaf blight was not identified, while locations designated as (+) represent fields in which northern corn leaf blight was present (continued).
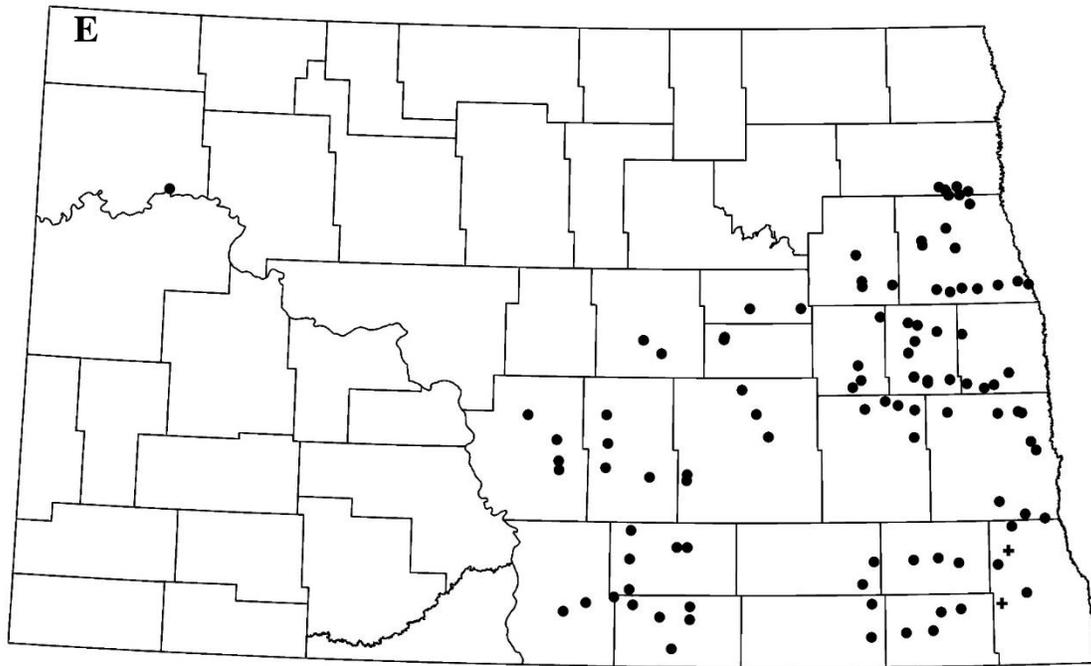
Figure A.3. Distribution of surveyed fields during the 2014 (A), 2015 (B), 2016 (C), 2017 (D), and 2018 (E) foliar disease survey of North Dakota corn fields. Locations designated as (•) represent fields where northern corn leaf blight was not identified, while locations designated as (+) represent fields in which northern corn leaf blight was present (continued).

APPENDIX B. GENETIC ANALYSES OF NORTH DAKOTA *CLAVIBACTER*

NEBRASKENSIS ISOLATES

Table B.1. *Clavibacter nebraskensis* isolates included in genetic analyses and number of variants when compared to *C. nebraskensis* reference genome NCPPB 2581 (NCBI accession NC_020891).

Year	Isolate	Region[z]	Number of variants
2011	ND Cmn 2011	SC	2885
2012	12-1504-A	SC	2075
2012	12-1626-A	EC	2531
2014	14-2-C	EC	1844
2014	14-21.1-A	SE	1830
2014	14-21.1-B #1	SE	1831
2014	14-21.2-A	SE	1819
2014	14-26-C	EC	2639
2014	14-26-E	EC	2754
2014	14-43-A	SE	1962
2014	14-61-A	SC	2464
2014	14-66-A	NC	2023
2015	15-7-F	EC	1675
2016	16-6-B	SC	2120
2016	16-9-G	EC	451
2016	16-11.1-B #1	C	1273
2016	16-11.3-B	C	454
2016	16-13-B #1	C	2473
2017	17-38-A	EC	1746
2017	17-69-A	EC	376

[z]North Dakota agricultural statistics districts (regions): EC = East Central, C = Central, NC = North Central, SC = South Central, and SE = Southeast.
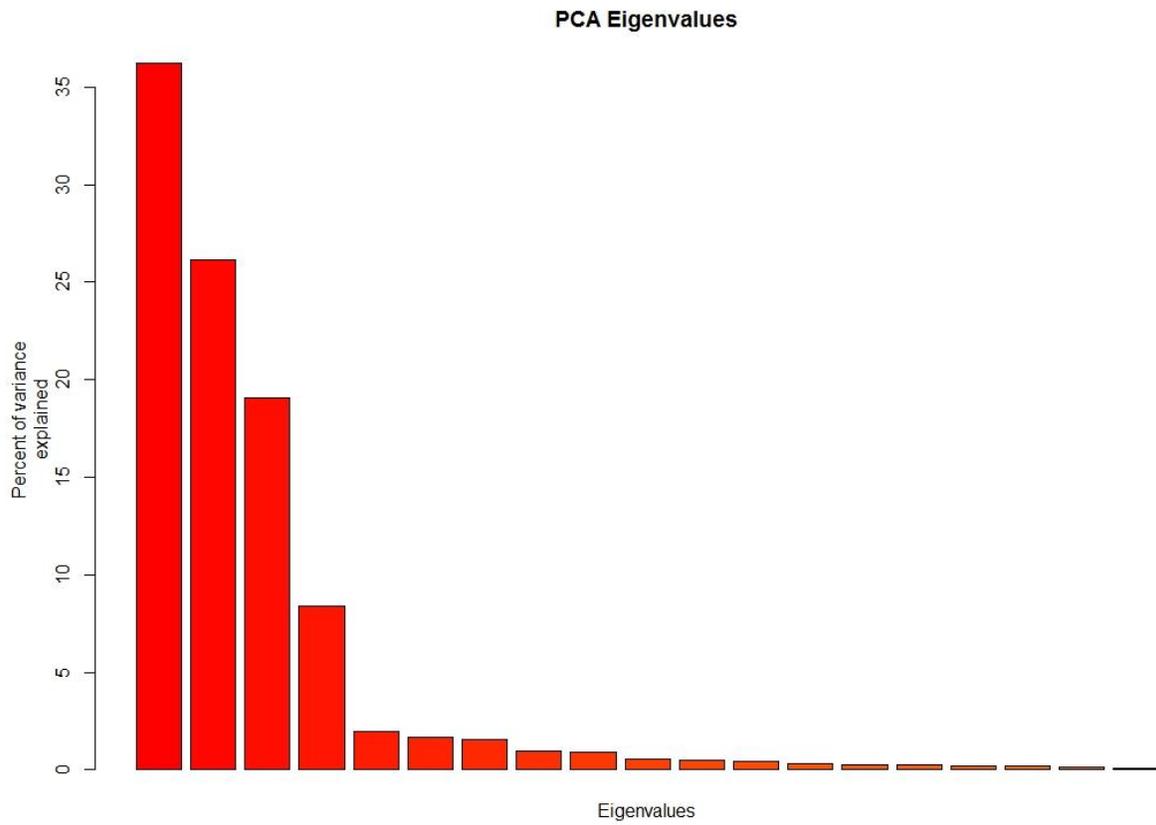
Figure B.1. Bar plot showing the variance explained by each principal components analysis (PCA).
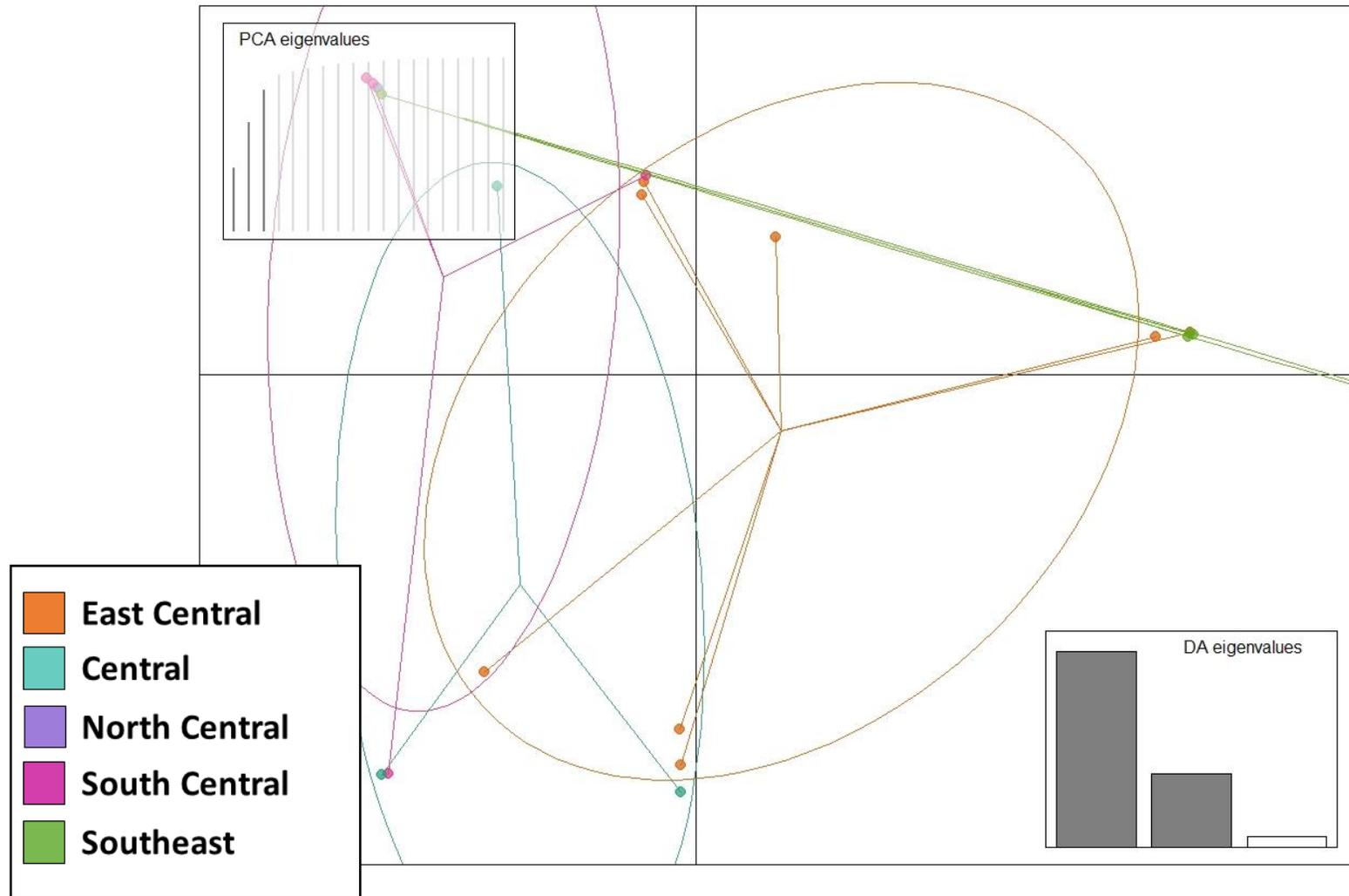
Figure B.2. Discriminant analysis of principal components of SNP data for 20 North Dakota (ND) *Clavibacter nebraskensis* isolates. Isolates are colored according to their ND agricultural statistics district (region) of origin. Regions are presented in the legend.
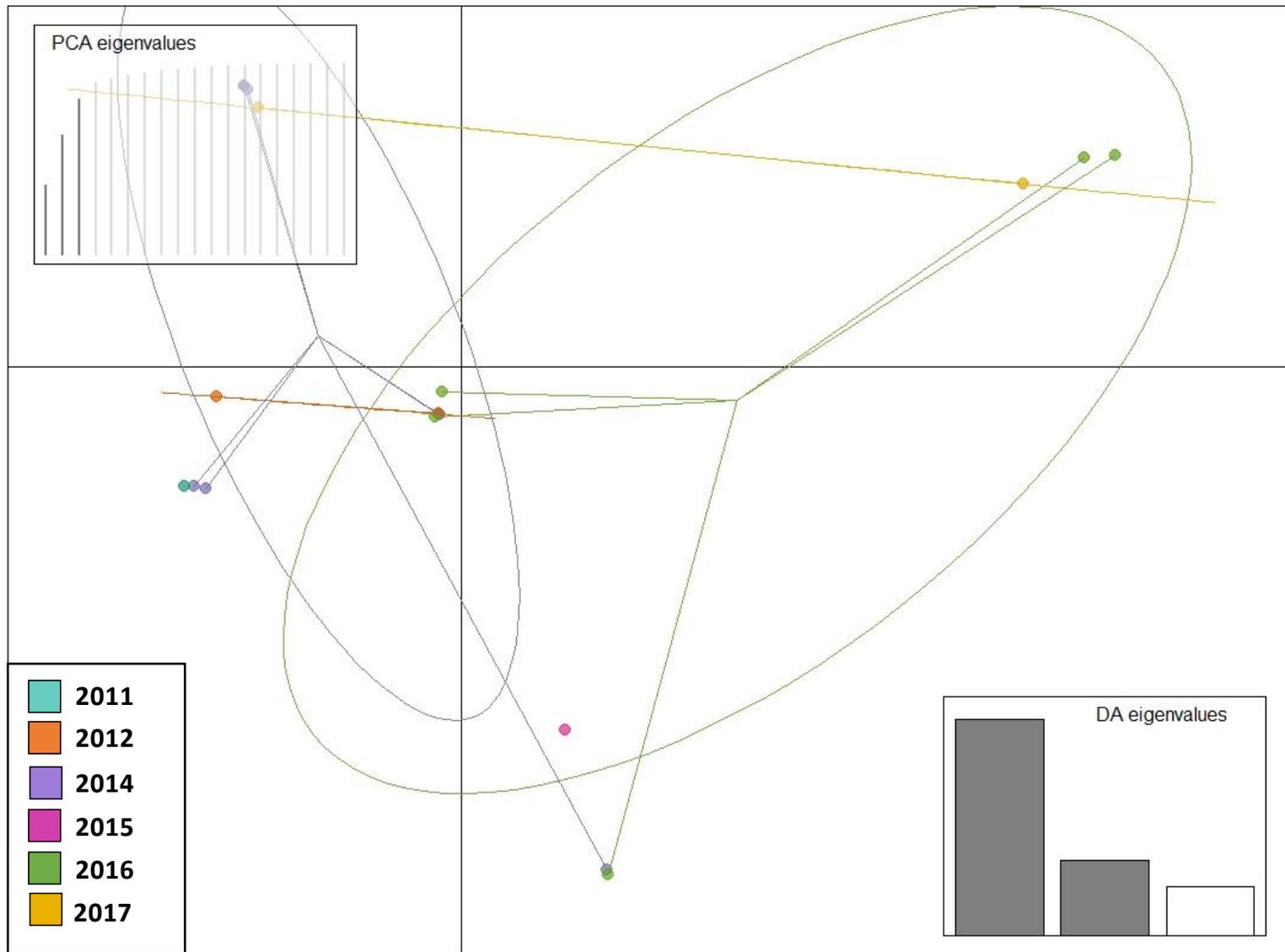
Figure B.3. Discriminant analysis of principal components of SNP data for 20 North Dakota *Clavibacter nebraskensis* isolates. Isolates are colored by their year of collection, which are presented in the legend.