THE ROLE OF ALTERNATIVE HOSTS AND HERBICIDES IN THE MANAGEMENT OF *CLAVIBACTER NEBRASKENSIS*, CAUSAL AGENT OF GOSS'S WILT OF CORN

by

Joseph Thomas Ikley

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STATEMENT OF COMMITTEE APPROVAL

Dr. William G. Johnson, Chair

Department of Botany and Plant Pathology

Dr. Kiersten A. Wise, Chair

Department of Botany and Plant Pathology, Adjunct

Dr. Steven G. Hallett

Department of Horticulture and Landscape Architecture

Dr. Stephen C. Weller

Department of Horticulture and Landscape Architecture, Emeritus

Approved by:

Dr. Christopher J. Staiger

Head of the Graduate Program

For all the people that put up with me over the years so that I can finally say "I'm a Dr, not a…"

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ABSTRACT

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Institution: Purdue University
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Committee Chair: William G. Johnson, Kiersten A. Wise

The reemergence of Goss's wilt of corn in the western Corn Belt in 2006, along with subsequent identification of the disease in 16 states, has led to renewed interest in the disease and its epidemiology. Goss's wilt, caused by the bacterium *Clavibacter nebraskensis*, is currently the third-leading cause of yield loss in corn from diseases in the United States. Its impact is exacerbated by the fact that cultural control methods are the only current means for its control. The objectives of our research were to (1) determine the role that alternative hosts of the bacterium play in the disease cycle and epidemiology of Goss's wilt, and (2) determine if postemergence herbicide use affects disease severity. Through a greenhouse experiment, we discovered three new weedy alternative hosts of the disease. In a series of field and greenhouse experiments, we found that *C. nebraskensis* can overwinter on alternative host and corn debris in Indiana. We found that *C. nebraskensis* did not become seed-borne in alternative hosts. In contrast to corn, no systemic infections were observed on alternative hosts, with the bacterium being restricted to inoculated leaf tissue. Using herbicides to control *C. nebraskensis*-infected weeds did not reduce the pathogenicity of the bacterium recovered from treated plants. The use of nicosulfuron, dicamba plus diflufenzopyr, and a 2X rate of glyphosate postemergence increased disease severity in one experiment, but postemergence herbicides did not influence disease severity in a second experiment. Corn yield was not affected. This indicates that herbicide use may play a role in the epidemiology of Goss's wilt in some years, but ultimately corn yield is not affected. Our results demonstrate that the host range of *C. nebraskensis* is wider than previously thought, and that postemergence control of alternative hosts may not be sufficient in reducing inoculum levels. Our results suggest that failure to control alternative hosts could negate some of the benefits of crop rotation to reduce inoculum levels in a field, thus playing an important role in the epidemiology of Goss's wilt.

CHAPTER 1. LITERATURE REVIEW

1.1 Occurrence, Spread, and Symptoms of Goss's wilt

During the summer of 1969, University of Nebraska extension personnel were asked to examine a corn field in Dawson County, Nebraska that was exhibiting unique symptoms. Attempts to isolate the causal agent of these symptoms revealed a new bacterial pathogen that was originally classified as *Corynebacterium nebraskense*, and was later reclassified as *Clavibacter nebraskensis* (Vidaver and Mandel 1974; Li et al. 2018). The disease was initially named Leaf freckles and wilt, but was later renamed Goss's wilt after former University of Nebraska Plant Pathology department head Robert Goss (Schuster 1970). Goss's wilt has become the accepted name of the disease, though it is still sometimes referred to as Leaf freckles and wilt in the literature.

Corn fields with symptomatic plants were confirmed in three Nebraska counties in 1969. In 1970, the disease was detected in 9 counties throughout the state. The disease was found in western Iowa in 1971, and northern Kansas in 1972 (Wysong et al. 1973). By 1974, the disease was widespread through Nebraska, and found in one or more counties in Colorado, Iowa, Kansas, and South Dakota (Schuster 1975). By the end of the 1970's the disease was also found in Wyoming and was an annual occurrence across Nebraska. Then from the mid 1980's until 2006, only sporadic cases of the disease were reported, and usually on plants exhibiting physical injury (Jackson et al. 2007). Then in 2006, the disease was reportedly widespread across western Nebraska, eastern Colorado, and Southeastern Wyoming (Jackson et al. 2007). Since 2006, the disease has been reported in several new states that redefined the geographic boundaries of the disease. It is now been found as far east as Indiana, and as far south as Louisiana and the panhandle of Texas (Ruhl et al. 2009;

Korus et al. 2011; Singh et al. 2014). The disease has been reported as far north as Minnesota and North Dakota (Malvick et al. 2010; Friskop et al. 2014). In total Goss's wilt has been reported in 16 states in the United States (US): Colorado, Illinois, Indiana, Iowa, Kansas, Louisiana, Minnesota, Missouri, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas, Wisconsin, and Wyoming (Friskop et al. 2014; Hosack et al. 2016; Jackson et al. 2007; Korus et al. 2011; Malvick et al. 2010; Ruhl et al. 2009; Schuster 1975; Singh et al. 2015; Wysong et al. 1973; Yasuhara-Bell et al. 2016). Goss's wilt has also been reported in the Canadian provinces of Alberta, Manitoba, and Ontario (Desjardins 2010; Howard et al. 2015; Tambong et al. 2015).

1.2 Biology and Ecology

C. nebraskensis is a Gram-positive bacterium, which is a property that initially helped identification of the causal agent of Goss's wilt from *Erwinia sterwartii*, which produces similar symptoms on corn with the disease Stewart's wilt (Vidaver and Mandel 1974). *C. nebraskensis* is a non-motile oxidative-negative, catalase-positive, club shaped rod the averages 0.5 to 2.5 µm in length (Jardine and Clafflin 2016). Colonies of the bacterium are orange-pigmented, circular, convex, glistening, and butyrous with entire margins when plated on Nutrient Broth-Yeast (NBY) media (Vidaver and Mandel 1974).

The optimal temperature for growth on NBY is between 24 C and 28 C (Vidaver and Mandel 1974), with the mean temperature for optimal growth being 27 C (Smidt and Vidaver 1986). The pathogen will grow rapidly between 16 C and 32 C, with retarded growth occurring at colder temperatures and pathogen death occurring at 38 C (Smidt and Vidaver 1986). Gross and Vidaver (1979) developed a semi-selective medium to help isolate and quantify the bacterium when recovered from field samples. They named the

medium *Corynebacterium nebraskense* selective medium (CNS). This medium proved useful in isolating *C. nebraskensis* while greatly reducing the number of Gram-negative bacterium and fungi that can be contaminants on NBY.

1.3 Survival and Infection

C. nebraskensis overwinters and survives on plant debris and both on and within corn seed. The bacterium can survive on corn leaf, stem, cob, and ear tissue on the soil surface for up to 10 months (Schuster et al. 1972). The bacterium cannot survive that long on leaf, cob, and ear tissue when buried 20 cm deep, but it can survive on corn stems at those depths. At a depth of 10 cm, the bacterium only survived on stems and cobs. Pure colonies of the bacteria did not survive at any depth in the soil profile (Schuster et al. 1972).

Studies have also revealed that *C. nebraskensis* can survive in and on corn seed. The pathogen has been documented to infest seed at 11 to 31 % (Biddle et al. 1991; Schuster 1975). The bacterium was isolated near the embryo and between the scutellum and the endosperm (Schuster 1975). This infestation rate translates to a very low rate of seed-to-seedling transmission of 0.4 to 1.6 % (Biddle et al. 1991; Schuster 1975). The difference in the number of plants exhibiting seed-to-seedling transmission compared to the number of seed confirmed to contain *C. nebraskensis* was attributed to poor germination of seed with high populations of the bacterium.

Corn debris was determined to be the primary source of inoculum by Schuster (1975). He collected leaves from infected fields in the spring of 1970 following the first reports of the disease in 1969. Pathogenic *C. nebraskensis* was recovered from leaf tissue in these fields for 5 consecutive years. Schuster (1975) also took infected stubble and placed it in small plots in a field with no history of the disease. The disease severity in plots

with infected stubble vs control plots was 44% vs 8 % in September 1971. Recently, Eggenberger et al. (2016) found that infected corn debris contributes more to the spread of Goss's wilt than bacterial spread from living corn plants infected with *C. nebraskensis*.

Severe Goss's wilt infection is typically associated with injured corn plants (Jardine and Claflin 2016). Physical wounding sufficient to cause *C. nebraskensis*-infection can occur through hail, sand-blasting, heavy rain, and high winds (Rocheford et al. 1985). The bacterium can be transported from a source of inoculum to the wound through water via rain splash or overhead irrigation. *C. nebraskensis* has been documented to live epiphytically on leaves of a susceptible popcorn hybrid, indicating that the bacteria may already be present on the leaf at time of wounding. Recent research by Mallowa et al. (2016) indicates that wounding may not be required and that *C. nebraskensis* can enter the corn leaves through stomata at rates of 20 to 30 %. They also reported that symptoms were more severe in higher humidity environments than lower humidity environments. Once inside the leaf tissue, *C. nebraskensis* can persist on the vascular tissue or move systemically through vascular bundles (Schuster 1975). Mbofung et al. (2016) reported that initial colonization occurs similarly in both resistant and susceptible corn hybrids. *C. nebraskensis* moves between corn cells by disrupting the cell walls. Then in susceptible hybrids, spread continues by disrupting cell walls in the xylem. Resistant hybrids produce a dense matrix in the xylem that deforms and restricts the movement of the bacteria within the plant.

The leaf blight phase of the disease exhibits symptoms that can be easily confused with other disease and abiotic stresses. When several lesions coalesce, the damage can often be confused with drought stress or nitrogen deficiency (Treat and Tracy 1990).

Typical symptoms include plant leaf tissue with large necrotic areas that are preceded by water-soaked lesions. This is very similar and easily confused with symptoms of Stewart's wilt (Schuster 1975). Unique to Goss's wilt infections, there are typically small dark green to black irregular areas embedded in the watersoaked lesions that resemble freckles (Jardine and Claflin 2016). Other common symptoms can include bacterial exudate that makes the leaf appearance shiny, as well as buggy-whipping in severe infection in small plants (Calub et al. 1974c; Schuster 1975). Systemically infected plants can wilt and die and vascular tissue can have an orange color. A wet stalk rot can also occur with systemic infections (Rocheford et al. 1989).

1.4 Inoculation Techniques

Several inoculation techniques have been used while conducting research with *C. nebraskensis*. The first method reported was a cut and spray method that involved cutting off the leaf tip and spraying inoculum on the remaining tissue (Schuster et al. 1972). Calub et al (1974c) developed three methods to inoculate corn plants with *C. nebraskensis*. Two methods involved a pin prick device. The first had a sponge containing the inoculum at the terminal end of a device that pricked holes in the leaf. The second method pricked holes in the leaf and inoculum was sprayed on. The third method involved directly injecting inoculum into the corn stem. All methods produced consistent disease symptoms (Calub et al. 1974c). Korus (2011) developed a technique where the end of the corn leaf was cut off, and the remaining leaf was dipped directly into inoculum. Recently Soliman et al. (2018) developed a method that attempts to standardize the amount of inoculum exposed to corn plants. They removed a 5 mm diameter circle of the upper leaf epidermis and deposited 1 ml of inoculum onto the wound. This resulted in a consistent wound and amount of

inoculum delivered. Corn plants tended to show more consistent and severe symptoms when inoculated at the V3-V5 corn stages (Calub et al. 1974b). Suparyono and Pataky (1989b) were able to detect differences in yield loss based on inoculation stage, with more severe yield loss occurring at V3-V5 growth stages compared to V5-V7 inoculations. Several papers also use a mixture of isolates in order to accurately represent pathogen population variability (Schuster et al. 1972).

1.5 Detection and Isolation Techniques

A semi-selective medium was developed in order to recover and isolate *C. nebraskensis* from corn (Gross and Vidaver 1979). Smidt and Vidaver (1986a) found that LiCl was toxic to certain isolates of *C. nebraskensis*, so that ingredient is typically left out of CNS. Other bacteria that are not pathogenic to corn can grow and outcompete *C. nebraskensis* on CNS, thus population levels may be underestimated during sampling (Smidt and Vidaver 1986b). Corn itself has been used as a recovery medium when testing overwintered debris for the presence of pathogenic *C. nebraskensis* (Schuster 1975). Korus (2011) tested several commercially available diagnostic kits developed for *Clavibacter michiganensis* to determine if they could successfully identify *C. nebraskensis*. He found that an ELISA test kit,(Neogen®) and Agdia ImmunoStrips® could positively identify *C. nebraskensis* isolated from corn or from agar plates. These tests could not consistently differentiate the *Clavibacter* species tested, meaning that symptomology on hosts remained one of the best methods to differentiate the *Clavibacter* species. Several tests have been developed recently that improve the ability to detect *C. nebraskensis* from other *Clavibacter* species. Yasuhara-Bell et al. (2016) developed a loop-mediated amplification (LAMP) assay to detect tripartite ATP-independent periplasmic (TRAP)-type C4-dicaroxylate transport

system that is unique to *C. nebraskensis*. Tambong et al. (2016) developed a TaqMan Real-Time PCR for specific detection of *C. nebraskensis*. McNally et al. (2016) developed primers targeting CMN_01184, a gene that is specific to *C. nebraskensis*. The detection limits were 30 and 3 ng of pure *C. nebraskensis* DNA and 100 and 10 CFU for conventional PCR and qPCR, respectively. These three newer detection and identification techniques are specific to *C. nebraskensis*, and will improve speed for positive confirmation and should help reduce false positive results when testing for *C. nebraskensis*.

1.6 Pathogen Variability

Smidt and Vidaver (1987) noted variability in the pathogen within a single popcorn field. They detected 4 different isolates based on colony morphology. With such a widespread geographical range of the disease over the last 4 decades, some genetic variability is to be expected. Agarkova et al. (2011) examined the population structure of 131 *C. nebraskensis* isolates collected between 1969 and 2009. They determined that *C. nebraskensis* genetic diversity has increased recently, with 18.8 % of all isolates collected after 1999 separating genetically from isolates collected prior to 1999. There were also several isolates analyzed in that study that are non-pathogenic on corn. *C. nebraskensis* isolates have shown the potential to lose pathogenicity over time in storage. This has made it difficult to compare isolates across the years. This was first documented in the 1970's when an isolate lost its pathogenicity after repeated subculturing on media (Schuster et al. 1975). When the pathogen was stored in distilled water plus mineral oil and stored at 10 C, as well as when infected leaf samples were stored at room temperature (25 C), the isolates remained pathogenic for 23 months. Vidaver (1977) was able to maintain pathogenicity on NBY stored at 6 C, which proved more effective at maintaining pathogenicity than on NBY

stored at room temperature. She also reported that lyophilization remained the most consistent method for retaining pathogenicity. Schuster et al (1975) tried several methods to restore pathogenicity to isolates that had lost it, but no method was successful.

1.7 Inheritance of Resistance

Many corn hybrids have high levels of resistance to *C. nebraskensis*, though none exist that are truly immune to the pathogen (Jackson et al. 2007). Calub et al. (1974a) examined 113 different corn lines and reported a wide range of reactions to *C. nebraskensis*. There were some lines that were highly resistant, and some that were very susceptible, with most genotypes falling somewhere in between. Mbofung et al. (2016) reported that resistance in corn plants is due to a dense matrix in the xylem that restricts bacterial movement within corn vascular tissue. Susceptible hybrids do not produce this matrix, and *C. nebraskensis* is able to disrupt cellular walls and spread through the xylem. The few studies that examined inheritance of resistance believed it to be controlled by only a few genes and suspect it to be quantitative (Carson and Wicks III 1991; Martin et al. 1975). Additive gene action is of primary importance in inheritance of resistance to Goss's wilt (Ngong-Nassah et al. 1992). It has been concluded that resistant and susceptibility to Goss's wilt are at least partially dominant since resistant inbred parents contribute resistance to F1's, and the same is true of susceptible parents. The crosses of corn lines with intermediate resistance to Goss's wilt produced more variable results in F1 generations (Ngong-Nassah et al. 1992). It has been over 20 years since literature examining the inheritance of resistance to *C. nebraskensis* has been published. With improvements in genetics in the last two decades, there is potential for finding more definitive answers regarding resistance to *C. nebraskensis*. Ikley et al. (2015) reported that the alternative hosts johnsongrass and

shattercane have a hypersensitive-type response to *C. nebraskensis* that restricts spread of the bacterium after initial colonization. Further research is required to elucidate the mechanism of response and determine if it could be inserted into corn to help with management of Goss's wilt.

1.8 Yield Loss and Management

Yield losses up to 44 to 50% have been documented in controlled studies in field corn (Carson and Wicks III 1991; Jardine and Claflin 2016). Jackson et al. (2007) reported a yield difference of 63% in one field with both a susceptible and tolerant hybrid planted in it. Greater losses have been reported in specialty corn. Pataky et al. (1988) reported a yield loss of 68% in sweet corn. Suparyono and Pataky (1989a) reported losses of up to 95% of marketable sweet corn ears due to Goss's wilt. They also showed reduction in ear diameter up to 44% and ear length up to 50%. In Indiana, losses of 60 bushels per acre were observed between fields with a similar history and hybrid of popcorn planted, with the major difference being one field was infected with *C. nebraskensis* (Wise et al. 2010). Across the Midwestern US and Ontario, Canada, Goss's wilt was the third-leading cause of yield loss due to disease between 2012 and 2015 when it caused an estimated loss of 13 million metric tons (Mueller et al. 2016).

There are currently no effective chemical management options for control of Goss's wilt. Schlund (2015) evaluated copper hydroxide, several commercially available fungicides, and pesticide adjuvants and found they had no effect on Goss's wilt severity. Langemeier et al. (2017) found a correlation between fields with a history of Goss's wilt and fields that had glyphosate applied to them, but the authors speculated it was due to widespread use of glyphosate in the Corn Belt. Williams et al. (2015) found that Goss's

wilt incidence was independent of glyphosate application, and was caused by a corn hybrid's susceptibility to *C. nebraskensis*. Cultural control methods remain the best strategies to manage the disease. Jackson et al. (2007) contributes an increase in no-till production, loss of hybrids with high levels of resistance, and continuous corn in some fields for up to 50 years for the reemergence of Goss's wilt in 2006. Langemeier et al. (2017) found that fields with corn planted with susceptible hybrids at populations greater than 67,500 seed per hectare were correlated with having Goss's wilt. Campbell (2017) found that planting popcorn in Indiana led to more incidence of Goss's wilt than fields planted with field corn. Together, these studies imply that tillage to reduce crop residue, crop rotation to a non-host crop, and utilizing hybrids with high resistance are the main tactics to manage this disease.

There are several alternative hosts that have been identified in the literature. Schuster et al. (1972) found that shattercane [*Sorghum bicolor* (L.) Moench ssp. *Arundinaceum* (Desv.) de Wet & Harlan], green foxtail [*Setaria viridis* (L.) Beauv], eastern gamma grass [*Tripsacum dactyloides* (L.) L.], sugarcane (*Saccharum spontaneum* L.), teosinte (*Euchlaena mexicana* Schrad), sudangrass [*Sorghum bicolor* (L.) Moench ssp. *drummondii* (Nees ex Steud.) de Wet & Harlan], and grain sorghum [*Sorghum bicolor* (L.) Moench ssp. bicolor] are hosts of *C. nebraskensis*. Wysong et al. (1981) claimed that the pathogen was isolated from barnyardgrass [*Echinochloa crus-galli* (L.) Beauv], though no evidence was presented in their abstract. Langemeier et al. (2014) confirmed that green foxtail is a host, and also found that [bristly foxtail *Setaria verticillata* (L.) Beauv], giant foxtail (*Setaria faberi* Herm), and yellow foxtail [*Setaria pumila* (Poir.) Roemer & J.A. Schultes] are hosts of the bacterium. Ikley et al. (2015) reported that nnual ryegrass (*Lolium*

multiflorum Lam.), large crabgrass [*Digitaria sanguinalis* (L.)], and johnsongrass [*Sorghum halepense* (L.) Pers.] were additional hosts. Most recently, woolly cupgrass [*Eriochloa villosa (Thunb.) Kunth]* and the native grass big blustem (*Andropogon gerardi Vitman)* and little bluestem [*Schizachyrium scoparium (Michx.) Nash]* have been confirmed as alternative hosts to *C. nebraskensis* (Webster 2016). While no experimental evidence has shown these alternative hosts to contribute to disease severity, control of alternative hosts could be a key management tool to reduce inoculum levels. Campbell (2017) found that giant foxtail, yellow foxtail, and large crabgrass were found in 94%, 74%, and 64% of fields in areas of Indiana where Goss's wilt is prevalent. He speculated that this was because these weeds are common in that area, regardless of the presence of *C. nebraskensis*. Typical disease symptoms were never found on alternative hosts during his survey. Nevertheless, control of alternative hosts could be of particular importance in crop rotation years to not allow a bridge host between corn crops.

1.9 Summary and Justification of Research

The reemergence and spread of Goss's wilt across the entire Midwest has been attributed to an increase in no-till production, an increase in corn-on-corn production, and reduced attention in breeding towards developing hybrids with high tolerance to the disease. Some studies have revealed alternative hosts to the pathogen. However, these studies only confirmed these weed species and native plants to be hosts of the bacterium, and their role in disease epidemiology has not been explored. To gain an understanding of the importance of these hosts, further studies must be conducted to evaluate the interaction of the bacterium with these alternative hosts.

C. nebraskensis has documented strains showing increased genetic variability in the last decade (Agarkova et al. 2011). Due to this increase in genetic variability, it is possible that the host range of this bacterium has changed. For this reason, it is worth re-testing some of the important weed species found to be non-hosts in the original host range evaluation conducted by Schuster (1975). It is also worth expanded testing of the host range of *C. nebraskensis* to include other important weed species not evaluated in that study. Due to the increasing popularity of cover crops, several species commonly used as cover crops are also important to test due to the number of hectares they are being planted on. This justification led to the first study conducted in this research that identified three new hosts of the disease: johnsongrass, large crabgrass, and the cover crop annual ryegrass (Ikley et al. 2015). The popularity of annual ryegrass as a cover crop drives the need to explore what role this grass may play in epidemiology of Goss's wilt.

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CHAPTER 2. ANNUAL RYEGRASS (*LOLIUM MULTIFLORUM*), JOHNSONGRASS (*SORGHUM HALEPENSE*), AND LARGE CRABGRASS (*DIGITARIA SANGUINALIS*) ARE ALTERNATIVE HOSTS FOR *CLAVIBACTER MICHIGANENSIS* SUBSP. *NEBRASKENSIS*, CAUSAL AGENT OF GOSS'S WILT OF CORN

2.1 Abstract

Goss's bacterial wilt and leaf blight of corn is caused by the bacterium *Clavibacter michiganensis* subsp. *nebraskensis* (Cmn). This disease has recently re-emerged as an important disease in the Midwestern United States (US) and continues to spread. Cultural practices are currently the only methods available for controlling the disease. Weedy species in the genera *Echinochloa*, *Setaria*, and *Sorghum* have previously been described as alternative hosts of Cmn. The objective of this research was to use an isolate of Cmn from the eastern Midwest to examine the host status of previously confirmed hosts, as well as test whether additional weedy or cover crop species are alternative hosts of the bacterium. Plants were inoculated with a suspension of 1 x 10^8 colony-forming units of Cmn per ml in a greenhouse experiment. Leaves were observed for typical symptoms of Goss's wilt seven days after inoculation. Pathogen presence was determined by observing bacterial streaming microscopically, and isolating Cmn from symptomatic plants. Putative colonies of Cmn were confirmed using morphological and molecular methods. Koch's Postulates were completed on populations of new plant species that showed symptoms. Results revealed three new hosts of Cmn: annual ryegrass, johnsongrass, and large crabgrass. In contradiction to previous reports, barnyardgrass was not a host of Cmn in this study. Results also confirm that giant foxtail, green foxtail, shattercane, and yellow foxtail are hosts of Cmn. These results redefine the known host range of Cmn and are important in

identifying additional sources of inoculum to improve our understanding of the epidemiology of Goss's wilt.

2.2 Introduction

Goss's bacterial wilt and leaf blight (Goss's wilt), caused by the Gram-positive bacterium *Clavibacter michiganensis* subsp. *nebraskensis* (Cmn), was first discovered in a hybrid corn field in south-central Nebraska in 1969 (Schuster et al. 1972). The disease continued to spread throughout the western Midwest in the 1970's. Sporadic cases were reported until 2006, when several cases of the disease were documented in western Nebraska, southeastern Wyoming, and eastern Colorado (Jackson et al. 2007). The re-emergence of the disease after 25 years was likely due to continuous corn-on-corn cropping practices, an increase in conservation tillage, which leaves more plant debris on the surface, and wide-spread planting of Cmn-susceptible hybrids (Jackson et al. 2007). Since re-emergence, the disease has continued to spread and has since been confirmed in at least 12 states in the United States (US): Colorado, Illinois, Indiana, Iowa, Kansas, Minnesota, Nebraska, North Dakota, South Dakota, Texas, Wisconsin, and Wyoming (Friskop et al. 2014; Jackson et al. 2007; Korus et al. 2011; Malvick et al. 2010; Ruhl et al. 2009; Schuster 1975; Wysong et al. 1973). Goss's wilt can cause up to a 44 percent yield loss in highly susceptible corn hybrids (Carson and Wicks III 1991). In 2013, Goss's wilt was estimated to cause yield losses of 7 million metric tons across the US, with most yield losses occurring in the Midwest (Mueller and Wise 2014). This ranked Goss's wilt as the third leading cause of yield loss in corn due to disease following seedling blights and Northern corn leaf blight across the US. Losses can be more severe in specialty markets, where 95 percent reduction of marketable sweet corn ears has been documented (Suparyono and Pataky 1989).

Cmn overwinters on infected plant debris, seed-coat, and within corn seed (Schuster 1975). Overwintered plant residue appears to be the primary source of inoculum (the part of the pathogen that initiates disease), while infected kernels that demonstrated seed-to-seedling transmission occurred at rates around 1 percent (Biddle et al. 1990). Initial studies on host range of Cmn found that eastern gamagrass [*Tripsacum dactyloides* (L.) L.], grain sorghum [*Sorghum bicolor* (L.) Moench ssp. bicolor], green foxtail, shattercane, sugarcane (*Saccharum spontaneum* L.), sudangrass [*Sorghum bicolor* (L.) Moench ssp. *drummondii* (Nees ex Steud.) de Wet & Harlan], and teosinte (*Euchlaena mexicana* Schrad.) all served as alternative hosts of the pathogen (Schuster et al. 1972). In contrast to the original findings of Schuster et al. (1972), Wysong et al. (1981) reported that Cmn was isolated from barnyardgrass, though no experimental evidence was provided. Langemeier et al. (2014) confirmed reports by Schuster et al. (1972) that green foxtail is a host for Cmn and identified bristly foxtail [*Setaria verticillata* (L.) Beauv.], giant foxtail, and yellow foxtail as new hosts of Cmn. This report of yellow foxtail as a host contradicts the findings of Schuster et al. (1972).

The ambiguity in the current literature concerning which species serve as alternative hosts of Cmn could be due to: (1) genetic variation in the pathogen over time and geography, or (2) genetic variation in plant species over time and geography. For instance, Agarkova et al. (2011) examined the population structure of 131 Cmn isolates collected between 1969 and 2009 and determined that Cmn genetic diversity has increased recently, with 18.8 percent of all isolates collected after 1999 separating genetically from isolates collected prior to that date. This increase in genetic diversity of Cmn may help explain the differences in host range between Schuster et al. (1972) and later studies.

Genetic diversity between weed populations in the western and eastern Midwest could also contribute to differences in host susceptibility to Cmn. Outcrossing weeds such as *Lolium* spp. have demonstrated high levels of genetic variation both within and among populations (Barrett 1982). Wang et al. (1995) examined the genetic variation of the self-pollinating species green foxtail, and found it is low in total genetic diversity, but genetic variation can be highly variable among populations, particularly those south of latitude 43.5° N in the US (approximately the Minnesota-Iowa border). Genetic diversity between populations of barnyardgrass and yellow foxtail tested in previous studies could potentially help explain the ambiguity in their role as alternative hosts of Cmn.

The re-emergence of Goss's wilt as an annual concern across the Midwest necessitates the importance of examining alternative sources of Cmn inoculum and their role in disease epidemiology. The objective of this study was to screen several common and problematic weeds for their potential to host Cmn. Different populations of several weed species previously tested by Schuster et al. (1972) were included in the screen to determine if differences in weed species populations, or changes over time, affect their susceptibility to the pathogen. Crabgrass and foxtail species were included due to their prevalence in fields with Goss's wilt (Langemeier 2012). Also included were several weeds identified as late-season escapes in Indiana soybean fields in a survey conducted by Johnson et al. (2004), to help determine if these species may contribute to Cmn inoculum levels. Alternative hosts that survive in a soybean [*Glycine max* (L.) Merr.] crop could reduce the effectiveness of crop rotation in management of Goss's wilt by acting as a bridge host between cropping years. Additionally, an increased interest and adoption in using cover crops has resulted in cover crops planted on 10.3 million acres nationally, and

600,000 acres in Indiana in 2012 [USDA-NASS 2014]. Therefore, we also tested several species typically used as cover crops to determine if they were alternative hosts of Cmn.

2.3 Materials and Methods

2.3.1 Species Selection.

Weed species previously identified as a host of Cmn or in the same genus as a host of Cmn were screened. These included barnyardgrass, giant foxtail, green foxtail, johnsongrass, shattercane, and yellow foxtail. Eastern black nightshade (*Solanum ptychanthum* Dunal) was tested since it is an alternative host of the closely related bacterium *Clavibacter michiganensis* subsp. *michiganensis* (Cmm), causal agent of bacterial wilt and canker of tomato (*Solanum lycopersicum* L.) (Thyr et al. 1975). While there has never been a documented case of hosts of other *Clavibacter michiganensis* subspecies being susceptible to Cmn, or hosts of Cmn being susceptible to other *Clavibacter michiganensis* subspecies, we included a host of Cmm in the experiment due to the potential changes in pathogen or host biology over time. Problematic weeds tested that had not previously been identified as hosts of Cmn included common ragweed (*Ambrosia artemisiifolia* L.), common waterhemp (*Amaranthus rudis* Sauer), giant ragweed (*Ambrosia trifida* L.), large crabgrass, and Palmer amaranth (*Amaranthus palmeri* S. Wats.). Species tested due to their utilization as cover crops were annual ryegrass and cereal rye (*Secale cereale* L.). A Cmn-susceptible corn hybrid (DKC55-09RIB Brand Blend, Dekalb® Corn, Monsanto Company, St. Louis, MO) was included as a control.

2.3.2 Inoculum Preparation.

A confirmed strain of Cmn that was isolated from Pulaski county Indiana in 2008 and stored in a 20 percent glycerol solution at minus 80 C was obtained from the Purdue Plant and Pest Diagnostic Laboratory (Ruhl et al. 2009). The isolate was grown on Nutrient Broth Yeast (NBY) media for 3 d at 24 C. Bacterial colonies were suspended in sterile double-distilled water (ddH_2O). Inoculum concentration was adjusted to a concentration of 1 x 10^8 colony-forming units (CFU) per ml with a Spectrophotometer (Beckman Coulter DU 530, Beckman Coulter, Inc., Brea, CA) set at 600 nanometers (nm). A concentration of 1 x 10^8 CFU per ml provides more consistent infection levels for distinguishing resistance levels in corn hybrids (Calub et al. 1974b).

2.3.3 Experimental Design and Inoculation

Plants were arranged in a randomized complete block design with three replications and repeated once. In each replication, there were 10 plants per species: 7 were inoculated with Cmn, and 3 served as non-inoculated controls. Plants were grown in the greenhouse with day/night temperatures of 29/22 C. Supplemental light was provided with 400 watt high-pressure sodium lamps to maintain 12-h photoperiod.

Corn, giant ragweed, johnsongrass, and shattercane were direct-seeded into 655-cm^3 cone-tainers (Deepot, Stuewe & Sons, Tangent, OR) filled with potting soil (Redi-Mix, Sun-Gro Redi-Earth Plug and Seedling Mix, Sun-Gro Horticulture, Bellevue, WA). Annual ryegrass and cereal rye were direct-seeded into 164-cm^3 cone-tainers (Ray Leach SC-10 Super Cell Cone-tainers; Stuewe & Sons, Tangent, OR) filled with potting soil. All other species were sown into 26 by 26 by 6 cm flats containing potting soil and transplanted into 164 cm^3 cone-tainers once two true leaves were present. Plants were watered daily and

fertilized weekly [Miracle-Gro® Water Soluble All Purpose Plant Food (24-8-16); Scotts Miracle-Gro Products Inc., Marysville, OH].

Plants were inoculated when monocots reached three visible collars, and dicots had three nodes present. The two youngest, fully-expanded leaves of each plant were inoculated. The leaf tip from one of the leaves was excised and dipped into the inoculum for 5 s. On the second leaf, 4 holes were poked into the leaf tip using sterile push-pins, then approximately 9 ml of the inoculum was sponged onto the wounded area. At 7 d after inoculation (DAI), lesion lengths on leaves that were cut and dipped into the inoculum were measured from the excise wound to the edge of the water-soaked, symptomatic tissue. Leaves that were inoculated using the second method were visually assessed for percent symptomatic leaf area of the whole leaf to measure disease severity. Non-inoculated controls were included in each replicate and treated as follows: (1) one plant was injured using the same methods as the inoculated plants, but was not exposed to inoculum, and (2) two plants were neither injured nor exposed to inoculum.

2.3.4 Pathogen Confirmation

Inoculated leaves were sampled for laboratory testing to confirm the causal agent of the source of symptoms after initial disease ratings were performed. The edges of symptomatic tissue for plants exhibiting symptoms were sampled, and leaf tissue from three plants for each inoculation technique, per replicate, were examined microscopically (100X) for bacterial streaming. To test if plants that did not show symptoms were asymptomatic hosts of Cmn, tissue adjacent to inoculated leaf tissue was sampled and examined as described above. Both inoculated leaves were examined for bacterial streaming, and both inoculated leaves from an additional 2 plants per replicate were rinsed with sterile ddH_2O then

cultured onto the semi-selective *Corynebacterium nebraskense* selective (CNS) medium minus LiCl (Smidt and Vidaver 1986) to isolate the colonizing bacteria. Due to limited bacterial growth on CNS, 7 d after initial plating, bacteria were single-colony streaked onto NBY to determine bacteria morphology. Bacteria were Gram tested using the KOH method, which was performed by mixing a single colony of bacteria in 0.1 ml of a 3 percent KOH solution for 60 s with a sterile loop, then slowly raising the loop from the solution. Cell walls of Gram negative bacteria will dissolve and release DNA into the solution causing the solution to become more viscous, while cell walls of Gram positive bacteria will remain intact and solution viscosity will not change (Fluharty and Packard 1967). Agdia Immunostrips® test kits (Agdia Inc., Elkart, IN) that are designed for detection of Cmm were used to test inoculated leaves from 3 plants per replicate and all control plants per replicate. Though designed to detect Cmm, the test strips have been confirmed to be effective in also detecting the presence of Cmn (Korus 2011).

Bacterial colonies that matched the description of Cmn isolated on NBY (apricot-orange in color, circular, convex, glistening, and butyrous delimited from a darker center zone; Vidaver and Mandel 1974) and those that did not match this description were identified through genotyping and phenotpying. For genotyping analysis, the 16S region of bacteria was amplified (~1480 bp) through polymerase chain reaction (PCR) using F27 and r1492 primers (Malvick et al. 2010). The Cmn isolate used to inoculate plants in this study was included as a positive control during testing. Based on these results, a subsample of positive matches from each replicate, as well as all isolates that did not match the positive control were DNA sequenced. PCR product was sent to the Purdue University Genomics Core Facility for High Throughput "Sanger" Sequencing. Similarities in

nucleotide sequences to known isolates of Cmn were accomplished using BLAST against two known strains of Cmn (NCBI GenBank AM410697.1 and U09763.1; Ruhl et al. 2009). Bacterial colonies that were sequenced were also sent to Microbe Inotech Laboratories, Inc. (St. Louis, MO) for phenotyping through BiOLOG® methods. This method of phenotyping subjects the bacteria to 95 carbon sources in a 96-well plate. Tetrazolium redox dies are included in each well that turns a well purple in the presence of increased respiration when bacteria utilize a carbon source. The phenotypic fingerprint of purple wells is then compared to a species library to identify bacteria to the species level (Bochner 1989). The isolates in this study were prepared by streaking bacteria onto BiOLOG® Universal Growth agar (BUG) and were then incubated at 30 C for 24 h. The samples were suspended in an inoculating fluid then loaded into a GEN III/FF microplate that was incubated at 30 C for 24 to 48 h. Plates were tested using an automated micro-plate reader and compared against the BiOLOG® Gen III/FF database for identification.

2.3.5 Koch's Postulates

Plant species that were identified as hosts of Cmn via morphological and genotypic testing were subjected to Koch's Postulates. This is a four step process to confirm causal agents of disease where (1) the suspected pathogen must be consistently associated with the diseased plants, (2) the suspected pathogen must be isolated in a pure culture and described, (3) the disease must be reproduced in a healthy plant with the isolated organism, and (4) the same pathogen must be re-isolated from the inoculated plant (Schumann and D'Arcy 2007). Positive alternative host identification was determined if isolates tested positive in every morphological and genetic test performed. Bacterial colonies isolated from these plants and identified as Cmn through colony morphology, Gram testing, and PCR assays

were prepared using the method previously described. Ten plants per species were inoculated using the same methods, and 7 DAI symptoms were rated and described. Three control plants per species were included. Symptomatic leaf tissue was sampled as previously described and single colonies were Gram tested using the KOH method and their morphology was described.

2.3.6 Statistical Analysis

Disease severity (percent) and lesion length (cm) ratings of plant species identified as hosts of Cmn were checked for normality and were not transformed. Data were subjected to ANOVA in order to compare susceptibility of plants identified as hosts of Cmn using the PROC GLM procedure in SAS 9.3 (SAS Institute Inc., Cary, NC). Plants not identified as hosts were left out of the analysis. There was a significant interaction ($\alpha = 0.05$) of both disease severity and lesion length between experiments, so data were analyzed separately. Means were separated using Tukey's pair-wise comparison test at $P \leq 0.05$.

2.4 Results and Discussion

2.4.1 Symptoms and Disease Severity

Symptoms typical of Goss's wilt: water-soaking with dark green to brown "freckling" on the edge of chlorotic lesions (Figure 1a), appeared on all inoculated corn leaves in both experiments, confirming inoculum pathogenicity. Control plants of each species that were wounded but not inoculated developed small necrotic lesions around the wound which did not spread (*data not shown*). Inoculated leaves of annual ryegrass, giant foxtail, green foxtail, johnsongrass, large crabgrass, shattercane, and yellow foxtail developed symptoms indicative of pathogen infection when compared to control plants, with plants within the

same genus producing similar symptoms. Disease symptoms that resembled Goss's wilt in corn, particularly the development of "freckling" symptoms, were observed in all eight species. All three foxtail species developed dark brown freckles located on the edge of and embedded in water-soaked and chlorotic tissue (Figure 1b). Large crabgrass typically developed large dark green freckling embedded in water-soaked lesions, which are typically a mix of light-green and dark red to yellow tissue (Figure 1c). Annual ryegrass typically developed dark brown necrotic lesions that were preceded by red tissue. In more severe infections, dark brown freckles run along the leaf veins ahead of necrotic tissue (Figure 1d).

Johnsongrass and shattercane symptoms on inoculated leaves are similar to the description that Schuster et al. (1972) provided for shattercane; where dark green to brown, and occasionally red freckles developed on the edge of and embedded in water-soaked lesions (Figure 1e). We also observed a hyper-sensitive response where a red lesion formed around symptomatic areas within 48 to 72 hours after inoculation and seemed to contain any further spread of pathogen colonization. No evidence of colonization was found outside of these areas when examined in the laboratory (*data not shown*).

Barnyardgrass, cereal rye, common ragweed, common waterhemp, eastern black nightshade, giant ragweed, and Palmer amaranth all produced no visual symptoms when inoculated with Cmn. The only visible change were small necrotic lesions near the wounds that were similar to those found on the control plants (*data not shown*).

There was a significant interaction ($P \leq 0.05$) between experiments for disease severity, where annual ryegrass, giant foxtail, and large crabgrass had less disease severity when compared to other species in experiment 1 vs experiment 2; while green foxtail had

higher disease severity when compared to other species in experiment 2 vs experiment 1 (Table 1). Corn had the longest symptomatic lesion during both experiments, with 9.9 cm and 8.7 cm in experiments 1 and 2, respectively. In both experiments, the lower quartile of corn plants had longer lesion lengths than the most severe lesion of any weed species (Figure 2). The foxtail species all had similar lesion lengths (Table 1). Large crabgrass had a similar lesion length as all three foxtails in experiment 1, and had similar lesion lengths as green foxtail and yellow foxtail in experiment 2, with a larger lesion than giant foxtail in experiment 2. Johnsongrass and shattercane had similar mean lesion lengths in both experiments, while the lower quartile of johnsongrass plants had longer lesions than the upper quartile of shattercane plants (Figure 2). Annual ryegrass had either the shortest or was no different from the shortest lesion in each experiment. There was more variability in experiment 1 leading to annual ryegrass having a higher maximum lesion length (5 cm) than four of the other species (Figure 2).

For visual ratings of disease severity, corn and at least one of the foxtails had the largest percentage of symptomatic tissue in each experiment. In experiment 1, corn, giant foxtail, and yellow foxtail had the highest percentage of symptomatic leaf tissue with 19, 10, and 17 percent severity, respectively; whereas in experiment 2, corn, green foxtail, and yellow foxtail had the highest disease severity ratings with 9, 12, and 7 percent severity, respectively (Table 1). The upper quartile of yellow foxtail was equivalent with corn's upper quartile in experiment 1, at 20 percent disease severity for each species; whereas the upper quartile of green foxtail was larger than that of corn in experiment 2, with 20 percent disease severity vs 13 percent, respectively (Figure 3). In both experiments, green foxtail and yellow foxtail were highly variable with each species having individual plants with a

higher percentage of symptomatic tissue than any single corn plant. This variability among foxtail species between experiments differs from results obtained by Langemeier et al. (2014), who were able to pool data from two experiments together and found that giant foxtail had similar disease severity as corn, followed by green foxtail then yellow foxtail. The differences in ranking of disease severity between our experiments and those conducted by Langemeier et al. (2014) could be attributed to genetic differences in populations of foxtail species and the isolates of Cmn used, as well as the susceptible corn hybrid chosen in each experiment.

After corn and the foxtail species; annual ryegrass, johnsongrass, large crabgrass, and shattercane all had similarly low percentages of symptomatic leaf area in each experiment. One of the leading reasons for varying results between lesion length and percent disease severity is that the foxtails, large crabgrass, and annual ryegrass had shorter, narrower leaves than corn, johnsongrass, and shattercane (*data not shown*). Thus, even though those weeds typically had shorter lesion lengths, symptomatic tissue represented a larger percentage of leaf area. While these species had low levels of infection compared to corn, each one had individuals with infection levels similar to or higher than the average percent symptomatic area of corn in each experiment (Figure 3).Through testing 113 individual lines, Calub et al. (1974a) found that there exists a highly variable range of responses between corn hybrids to Cmn. Without testing several different populations within each weed species, we speculate that the variability of certain individuals being more susceptible to Cmn is due to natural variation in genetics within a weed population when compared to the monoculture nature of the susceptible corn hybrid.

2.4.2 Pathogen Confirmation

Across both experiments, bacterial streaming was observed in all leaves of corn, johnsongrass, large crabgrass, shattercane, and yellow foxtail. All leaves except one of annual ryegrass, and all except two leaves of both giant foxtail and green foxtail tested positive for bacterial streaming. Across experiments, no leaves tested positive for bacterial streaming for barnyardgrass, cereal rye, common ragweed, common waterhemp, eastern black nightshade, giant ragweed, or Palmer amaranth (*data not shown*). For all species, all inoculated leaves tested with the Immunostrips were positive for Cmn. Cmn was also recovered on CNS medium for inoculated leaves of all species tested. In plants where no symptoms were observed, we determined that they are not asymptomatic hosts since no streaming was observed, indicating that bacteria did not colonize leaf tissue. Since Cmn did not colonize the leaf tissue, and it was recovered on CNS medium and detected through the use of Immunostrips, it appears the bacteria can live epiphytically for at least 7 d on the surface of these non-host plants. Epiphytic populations are described as those that are capable of living on plant surfaces (Hirano and Upper 1983). Several cases of phytopathogenic bacteria living epiphytically on nonhost plants are reported in the literature. *Xanthomonas phaseoli,* causal agent of bean common bacterial blight, has been documented to live on the surface of the weeds redroot pigweed (*Amaranthus retroflexus* L.), common ragweed, common lambsquarters (*Chenopodium album* L.), barnyardgrass, and black nightshade (*Solanum nigrum* L.) for up to 21 d (Cafati and Saettler 1980). *Pseudomonas syringae*, causal agent of bacterial brown spot of bean, has been documented to overwinter epiphytically on the leaves of hairy vetch (*Vicia villosa* Roth), and correlated to epidemics in subsequent years (Ercolani et al. 1974). The presence of Cmn living

epiphytically for 7 d in this study suggests that Cmn does not need to colonize plant tissue to survive under ideal environmental conditions in the greenhouse.

All bacteria recovered using CNS tested Gram positive using the KOH test (*data not shown*). PCR resulted in 97.5 percent of isolates producing the same band as the control isolate. Those positive PCR results that were sent for sequencing all resulted in 95 percent or greater matches when BLASTed against Cmn isolates AM410697.1 and U09763.1. Negative PCR results had matches of 78 to 94 percent when sequenced and BLASTed against the same Cmn control isolates, and were more closely matched to bacteria in different genera. The BiOLOG® analysis of isolates that were confirmed with PCR to be Cmn resulted in a 99.6 percent match to the species level (*Clavibacter michiganensis*). With the exception of seven samples, all negative (non-Cmn) results from both sequencing and BiOLOG® ID agreed in identifying isolates to the same genus. The non-*Clavibacter* bacteria recovered were in the genera *Achromobacter*, *Arthrobacter*, *Bacillus*, *Curtobacterium*, *Gracilibacillus*, *Microbacterium*, *Ralstonia*, and *Staphylococcus*. Since these bacteria were only recovered in 2.5 percent of isolations, the semi-selective medium CNS proved fairly consistent with recovering only Cmn (*data not shown*).

2.4.3 Koch's Postulates

Annual ryegrass, corn, giant foxtail, green foxtail, johnsongrass, large crabgrass, shattercane, and yellow foxtail all produced similar symptoms as described in Figure 1 in all ten plants exposed to inoculum. No control plants exhibited the described symptomology. Bacterial streaming was confirmed microscopically for all inoculated leaves and leaves were plated onto CNS where Cmn was recovered on each plate. After single colony streaking onto NBY, bacterial colonies typical of Cmn were observed.

After completion of Koch's Postulates, we identified annual ryegrass, johnsongrass, and large crabgrass as newly discovered alternative hosts for the bacterium. In contrast to reports by Wysong et al. (1981), barnyardgrass is reported here as a nonhost of Cmn. Wysong et al. (1981) stated that "The pathogen was also isolated from green foxtail, barnyardgrass, and shattercane under field conditions." Without completion of Koch's Postulates to confirm barnyardgrass as a host, and given that we observed Cmn living epiphytically on barnyardgrass in this study, Wysong et al. (1981) may have isolated epiphytic populations of Cmn and erroneously reported it as an alternative host of Cmn. Our confirmation of johnsongrass, large crabgrass, and yellow foxtail also contradict the original findings of Schuster et al. (1972) that reported those three plants as nonhosts of Cmn. This difference may be a function of the Cmn isolates used in the experiments. Though Agarkova et al. (2011) found that the isolates Schuster et al. (1972) used are genetically similar to isolates from Indiana, the increased variability of Cmn populations isolated after 1999 in that study indicates that some changes have occurred since the pathogen was first discovered, which may affect the ability of certain isolates to cause disease on plants other than corn. The differences in reported hosts could also be due to a change in the biology of potential host plants, or due to population differences within tested species. This study also confirms results by Schuster et al. (1972) and Langemeier et al. (2014) that giant foxtail, green foxtail, shattercane, and yellow foxtail are alternative hosts of Cmn in populations in the eastern Midwest. Barnyardgrass, cereal rye, common ragweed, common waterhemp, eastern black nightshade, giant ragweed, and Palmer amaranth are not alternative hosts of Cmn. The identification of several important broadleaf weeds and barnyardgrass as nonhosts of Cmn indicates that these common weed escapes may not

reduce the effectiveness of crop rotation on management of Goss's wilt. Though these plants are nonhosts, the bacterium appears capable of living epiphytically on these species for at least 7 d. We did not quantify the epiphytic populations in this study, though the ability of bacterial colonies to increase while living epiphytically on nonhost plants has been reported for other bacterial species (Hirano and Upper 1983). More research is needed to determine population dynamics of epiphytic populations of Cmn to determine what role they may play in epidemiology of Goss's wilt.

The presence of weed hosts in fields with a history of Goss's wilt could potentially off-set the benefits of cultural control methods such as tillage and rotation to a non-host crop by serving as a bridge host between corn crops. With no current effective chemical control measures for Cmn, any tactic that can reduce the inoculum levels of Cmn can be a boon to Goss's wilt management. Control of these alternative hosts should be emphasized in fields at risk for Cmn for this reason.

Annual ryegrass is the first host species identified that has a winter annual growth pattern. Since an annual ryegrass cover crop is typically seeded in August or September and terminated in April or May, its use in areas with a history of Goss's wilt could provide a continuous living host for the pathogen throughout the winter months. While it is currently unknown what role an annual ryegrass cover crop may play in epidemiology of Goss's wilt, it is not recommended to plant a known host of Cmn in fields where Goss's wilt can be problematic. The finding that cereal rye is not a host of Cmn provides an alternate monocot cover crop that could be better suited for fields with Goss's wilt. Further research is needed to identify the importance of alternative hosts of Cmn in disease epidemiology and management.

2.5 Acknowledgements

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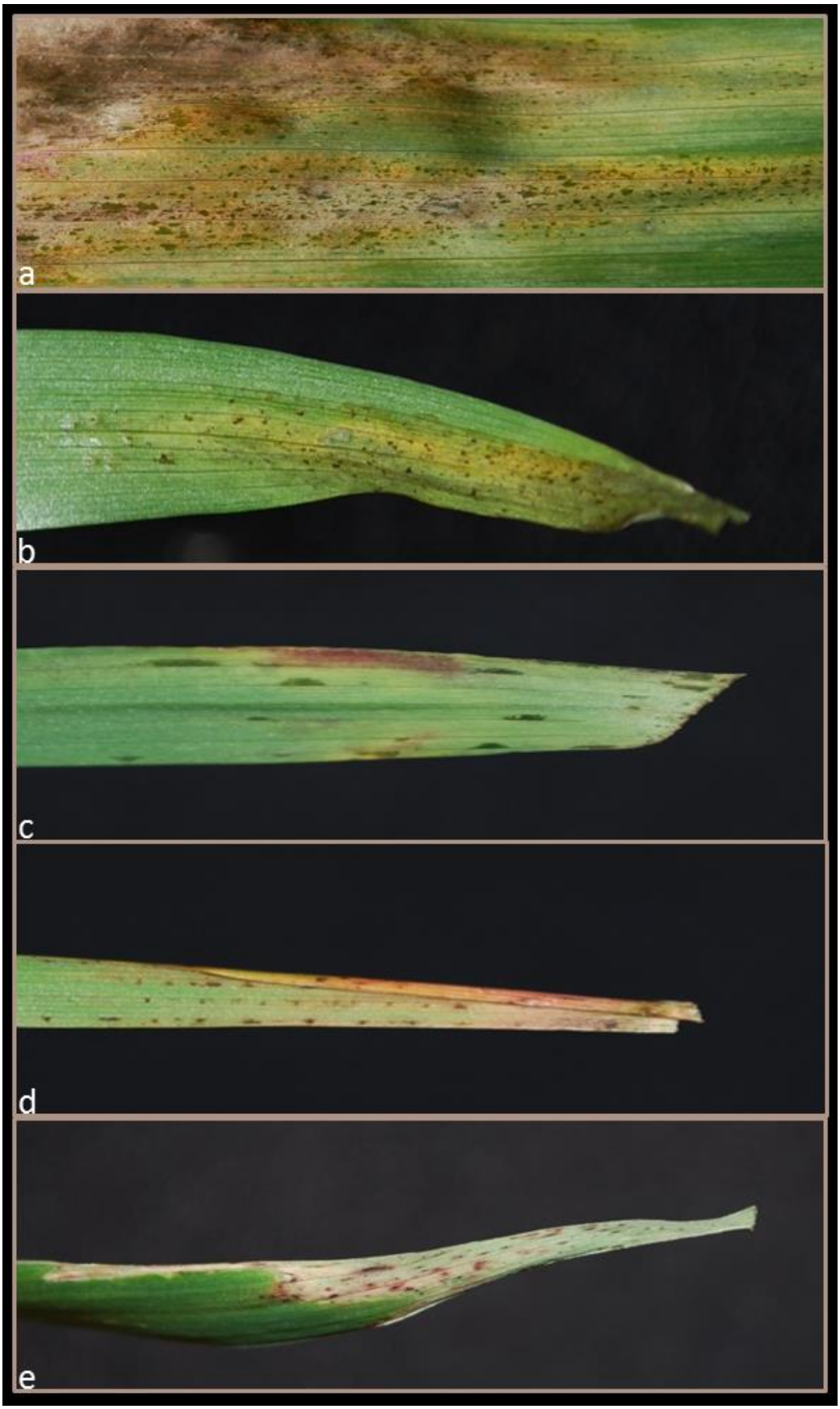
Table 2.1 Lesion length and disease severity of infection caused by *Clavibacter michiganensis* subsp. *nebraskensis* seven days after inoculation on host species.

Plant species	Lesion length[a]		Disease severity	
	Experiment 1	Experiment 2	Experiment 1	Experiment 2
	cm		%	
annual ryegrass	0.6 c	0.2 d	8 bc	3 c
corn	9.9 a	8.7 a	19 a	9 ab
giant foxtail	2.4 b	0.7 cd	10 ab	6 bc
green foxtail	1.6 bc	1.4 bc	7 c	12 a
johnsongrass	0.9 c	1.5 bc	6 c	4 c
large crabgrass	1.1 bc	1.9 b	10 bc	4 c
shattercane	0.3 c	0.4 cd	8 c	3 c
yellow foxtail	1.5 bc	1.2 bcd	17 ab	7 abc

[a]Mean values separated using Tukey's HSD. Values followed by different letters within column are significantly different ($P \leq 0.05$).

Figure 2.1. (A) Observed symptoms of Cmn-infection on corn. Small dark green to brown freckling occurring in water-soaked lesions on the edge of necrotic tissue. (B) Observed symptoms of Cmn-infection on giant foxtail. Dark brown freckling found on the edge of and imbedded in water-soaked and necrotic tissue. Symptoms are similar on green foxtail and yellow foxtail. (C) Observed symptoms of Cmn-infection on large crabgrass. Dark green freckling found imbedded in water-soaked and necrotic lesions. Lesions are often a mix of light green and crimson red in color. (D) Observed symptoms of Cmn-infection on annual ryegrass. Red to brown necrotic lesions that in severe infections are accompanied by dark brown freckling that runs along the leaf veins and precedes necrotic tissue. (E) Observed symptoms of Cmn-infection on johnsongrass. Dark green to brown, and occasionally red freckling found in water-soaked and necrotic tissue. Red lesions indicative of hyper-sensitive response form around symptomatic areas and contain the infection. Symptoms are similar on shattercane.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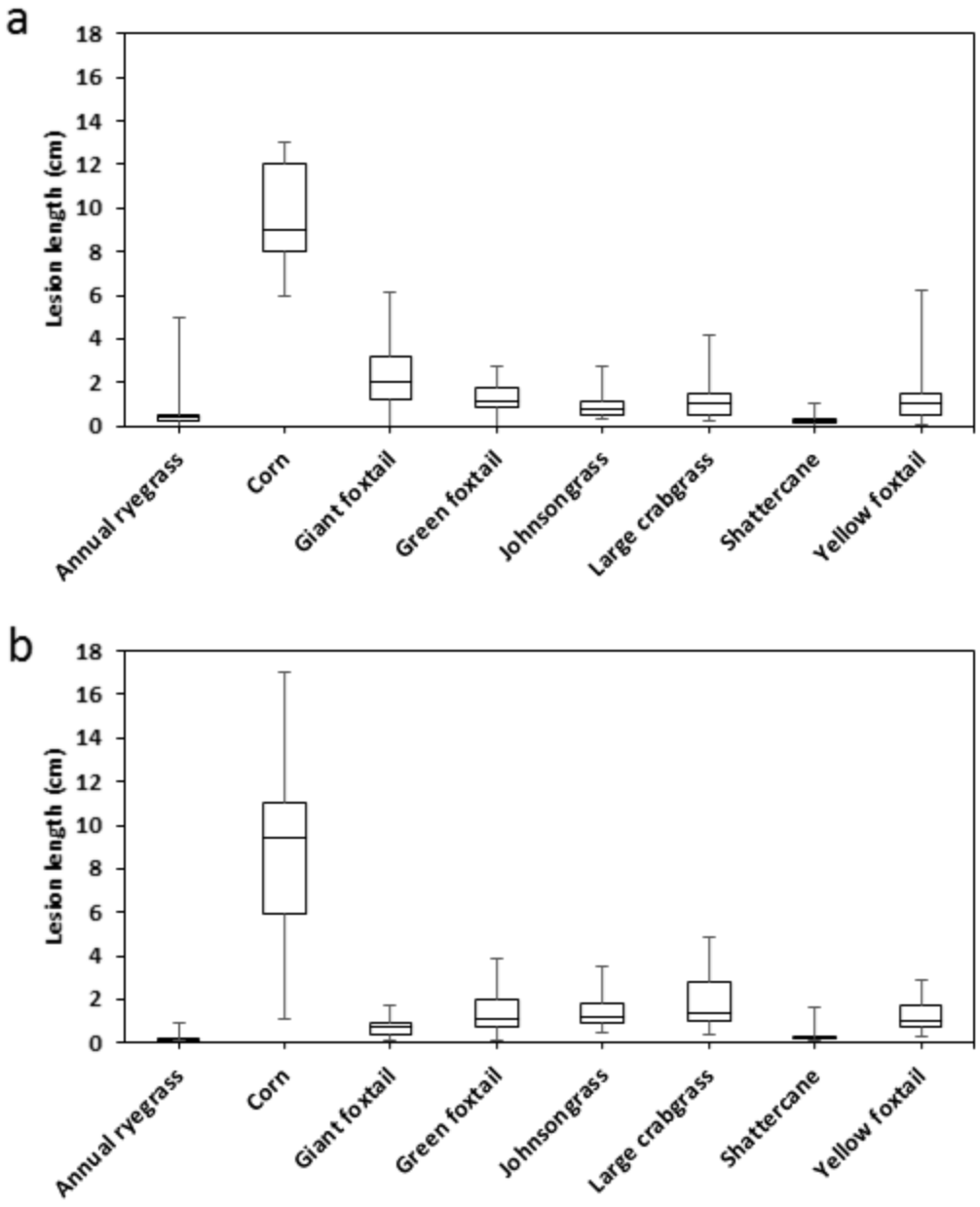

Figure 2.2. Box and whisker plots for symptomatic lesions on confirmed hosts of Cmn. Y-axis represents the length of lesions in cm of leaves that were inoculated by cutting the leaf tip and dipping the leaf into the inoculum in experiment 1 (A) and experiment 2 (B). The middle line is the median, the top of the box is the upper quartile, the bottom of the box is the lower quartile, and the whiskers extend to the maximum and minimum values.

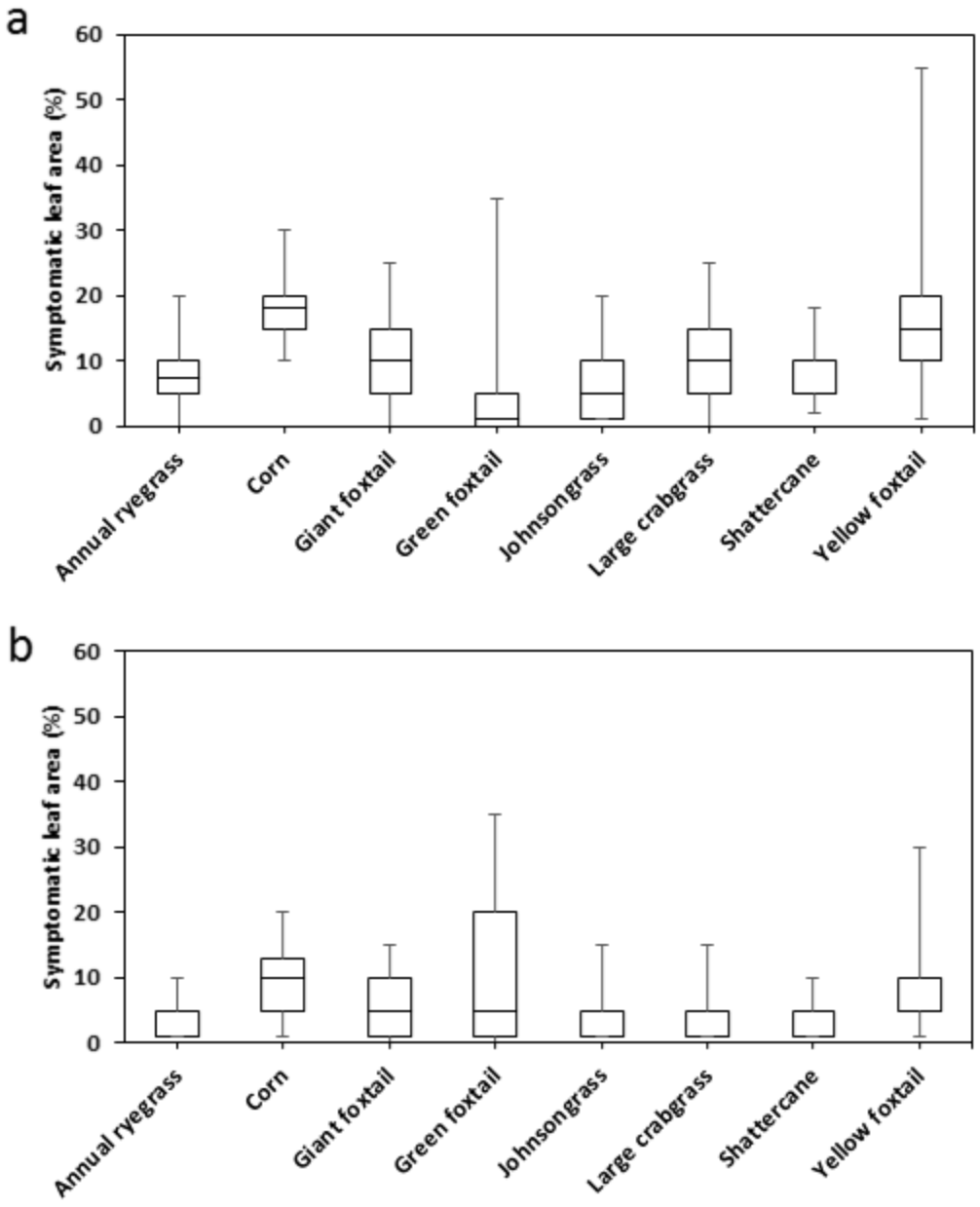

Figure 2.3. Box and whisker plots for symptomatic lesions on confirmed hosts of Cmn. Y-axis represents the percent symptomatic area of the leaf for leaves inoculated by poking holes into the leaf and sponging on the inoculum in experiment 1 (A) and experiment 2 (B). The middle line is the median, the top of the box is the upper quartile, the bottom of the box is the lower quartile, and the whiskers extend to the maximum and minimum values.

CHAPTER 3. EFFECT OF HERBICIDES ON RECOVERY AND PATHOGENICITY OF *CLAVIBACTER NEBRASKENSIS*, AND COMPARISON OF EFFICACY OF HERBICIDES ON WEEDS THAT ARE INFECTED WITH *CLAVIBACTER NEBRASKENSIS* COMPARED TO WEEDS THAT ARE NOT INFECTED

3.1 Abstract

Goss's bacterial wilt and leaf blight of corn is caused by the bacterium *Clavibacter nebraskensis*. This disease re-emerged as an important disease in the corn belt in the mid-2000's and has become more widespread with confirmed cases in 17 states in the Midwestern United States. Cultural practices are the only current method available to control this disease. Some grass weed species and cover crops have been documented as additional hosts of *C. nebraskensis*, therefore controlling these hosts could reduce inoculum levels in a field. The objectives of this study were to (1) apply single active ingredient herbicides to *C. nebraskensis*-infected weeds to determine if pathogenic *C. nebraskensis* could be recovered from plants after treatment, and (2) determine if there is a difference in efficacy of the same herbicides between weeds infected with *C. nebraskensis* compared to non-infected plants. In the greenhouse, three confirmed hosts were inoculated with a bacterial suspension containing 1 x 10^8 colony-forming units (CFU) of *C. nebraskensis* per ml. Treatments consisted of seven herbicides and an untreated control. For objective 1, leaf tissue from all plants were examined for bacterial streaming and plated onto *C. nebraskensis*-selective medium two weeks after herbicide treatment (WAT). Recovered bacteria were then used to inoculate a susceptible corn hybrid to test pathogenicity. Results show that herbicide treatment did not reduce pathogenicity of bacteria recovered from infected weed species. For objective 2, weed control was visually

estimated 1, 2, and 3 WAT, and dry weights were measured. Efficacy of glufosinate was reduced for *C. nebraskensis*-infected giant foxtail compared to non-infected giant foxtail. There were no differences for other herbicides tested between *C. nebraskensis*-infected weeds compared to non-infected weeds. The results from this study reinforce the need to control alternative hosts to avoid infection from *C. nebraskensis* that can lead to additional inoculum and reduced weed control.

3.2 Introduction

Goss's bacterial wilt and leaf blight (Goss's wilt) was first discovered in a hybrid corn (*Zea mays* L.) field in Nebraska in 1969 (Schuster et al. 1972). It is caused by the bacterium originally named *Corynebacterium nebraskense*, which has recently been re-classified as *Clavibacter nebraskensis* comb. nov. (Vidaver and Mandel 1974; Li et al. 2018). The disease was widespread in the western corn belt throughout the 1970's, but only sporadic cases were reported from the 1980's until the disease reemerged in western Nebraska, southeast Wyoming, and eastern Colorado in 2006 (Jackson et al. 2007). The re-emergence has been attributed to an increase in corn-on-corn cropping practices, an increase in conservation tillage, and widespread planting of susceptible corn hybrids (Jackson et al. 2007). Since re-emergence, the disease has been found in 16 states in the United States (US): Colorado, Illinois, Indiana, Iowa, Kansas, Louisiana, Minnesota, Missouri, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas, Wisconsin, and Wyoming (Friskop et al. 2014; Hosack et al. 2016; Jackson et al. 2007; Korus et al. 2011; Malvick et al. 2010; Ruhl et al. 2009; Schuster 1975; Singh et al. 2015; Wysong et al. 1973; Yasuhara-Bell et al. 2016). Goss's wilt caused an estimated loss of 13 million metric tons of corn yield in the Midwest and Ontario, Canada between 2012 and 2015, making it the third-

leading cause of yield loss in corn due to disease (Mueller et al. 2016). Yield losses up to 44 percent have been documented in field trials (Carson and Wicks III 1991). Losses can be more severe in specialty crops, where a 95 percent reduction in the number of marketable ears in sweet corn has been documented (Suparyono and Pataky 1989).

Schuster (1975) documented that *C. nebraskensis* overwinters on plant debris, on corn seed-coats, and within corn seed. Eggenberger et al. (2016) confirmed that infected corn residue is a primary source of inoculum. Infected kernels allow for long-distance transportation, but only demonstrate seed-to-seedling transmission at rates below 1 percent (Biddle et al. 1990). There are several known alternative hosts to *C. nebraskensis*, though the role of alternative hosts as an inoculum source is unknown. Commonly found weedy hosts of *C. nebraskensis* include bristly foxtail [*Setaria verticillata* (L.) Beauv.], giant foxtail (*Setaria faberi* Herm.), green foxtail [*Setaria viridis* (L.) Beauv.], yellow foxtail [*Setaria pumila* (Poir.) Roemer & J.A. Schultes], large crabgrass [*Digitaria sanguinalis* (L.)], shattercane [*Sorghum bicolor* (L.) Moench ssp. *Arundinaceum* (Desv.) de Wet & Harlan], and johnsongrass [*Sorghum halepense* (L.) Pers.] (Langemeier et al. 2014; Ikley et al. 2015). Ikley et al. (2015) found the commonly used cover crop annual ryegrass (*Lolium multiflorum* Lam.) is also an alternative host. A survey by Campbell (2017) found that giant foxtail, yellow foxtail, and large crabgrass were found in 94, 74, and 63 percent of fields, respectively, in regions of Indiana that have fields with a history of Goss's wilt. Giant foxtail and large crabgrass have also documented as common weed escapes in soybean fields across Indiana (Johnson et al. 2004). The prevalence of alternative hosts of *C. nebraskensis* in these fields is concerning due to their potential to increase inoculum within a growing season, and as an additional source of inoculum for future years.

In all published research, *C. nebraskensis* has been isolated from alternative hosts that were sampled while still alive and actively growing in either the greenhouse or field. It is unknown what effect treating *C. nebraskensis*-infected alternative hosts with herbicides has on pathogenicity of *C. nebraskensis* recovered from treated plants. Schlund (2015) tested several commercially available adjuvants, fungicides, and copper hydroxide for any effect on *C. nebraskensis*. She found that some adjuvants exhibit slight phytotoxic effects at field use rates *in vitro*, but found no effect on Goss's wilt severity when applied to *C. nebraskensis*-infected corn plants. A review by Duke et al. (2007) found limited data on the direct effects of herbicides on plant pathogens. They found twelve papers that reported direct toxic effects of herbicides on plant pathogens, however none of the reviewed papers tested the effects of herbicides on plant pathogenic bacteria.

The objectives of this study were to: (1) apply single active ingredient herbicides to *C. nebraskensis*-infected hosts to determine if pathogenic *C. nebraskensis* could be recovered from plants after treatment, and (2) determine if there is a difference in efficacy of those herbicides on *C. nebraskensis*-infected hosts compared to hosts that are not infected with *C. nebraskensis*. Giant foxtail and large crabgrass were selected to test due to their prevalence in fields with a history of Goss's wilt, and annual ryegrass was also selected due to its widespread use as a cover crop in the US (Campbell 2017; CTIC 2017).

3.3 Materials and Methods

3.3.1 Species and Herbicide Selection

We chose three grass species confirmed as alternative hosts of *C. nebraskensis* to use in these experiments (Ikley et al. 2015). We selected giant foxtail and large crabgrass, which are summer annual grasses that are commonly found weedy escapes in soybean fields in

Indiana (Johnson et al. 2004). We also chose annual ryegrass, which was planted as a cover crop on approximately 37,000 hectares in the US in 2017 (CTIC 2017).

Seven single active ingredient herbicides plus a no herbicide control were applied in each study. The herbicides and their respective rates used in this research are located in Table 3.1. These herbicides were selected due to their prevalent use in corn and/or soybean production in Indiana and the Midwest. Adjuvants were tank-mixed with herbicides based on instructions found on the herbicide labels (Table 3.1). Sources of adjuvants were as follows: Ammonium sulfate (AMS; N-Pak, Winfield Solutions, LLC, St. Paul, MN 55164) was added at 5% v/v, which resulted in 2 % w/v active ingredient AMS per tank mixture. Crop oil concentrate (COC; Prime Oil, Winfield Solutions, LLC, St. Paul, MN 55164) was added at 1% v/v. Methylated seed oil (MSO Ultra, Precision Laboratories, LLC, Waukegan, IL 60085) was added at 1 % v/v.

3.3.2 Inoculum Preparation

A confirmed strain of *C. nebraskensis* that was isolated from Pulaski county Indiana in 2008 and stored in a 20 percent glycerol solution at minus 80 C was obtained from the Purdue Plant and Pest Diagnostic Laboratory (Ruhl et al. 2009). This is the same isolate that was used by Ikley et al. (2015) in a host screen study. The isolate was grown on Nutrient Broth Yeast (NBY) media for 3 d at 24 C. Bacterial colonies were suspended in sterile double-distilled water (ddH_2O). Inoculum concentration was adjusted to a concentration of 1 x 10^8 colony-forming units (CFU) per ml with a Spectrophotometer (Beckman Coulter DU 530, Beckman Coulter, Inc., Brea, CA) set at 600 nanometers (nm). A concentration of 1 x 10^8 CFU per ml was selected in order to maximize the percent of infected tissue prior to herbicide application.

3.3.3 Inoculation of Weeds and Herbicide Treatment

Giant foxtail and large crabgrass were sown into 26 by 26 by 6 cm flats containing potting soil (Redi-Mix, Sun-Gro Redi-Earth Plug and Seedling Mix, Sun-Gro Horticulture, Bellevue, WA) and transplanted into 164 cm^3 cone-tainers (Ray Leach SC-10 Super Cell Cone-tainers; Stuewe & Sons, Tangent, OR) filled with unsterilized field soil once two true leaves were present. Annual ryegrass was direct seeded into 164-cm^3 cone-tainers filled with unsterilized field soil. Plants were watered daily and fertilized weekly [Miracle-Gro® Water Soluble All Purpose Plant Food (24-8-16); Scotts Miracle-Gro Products Inc., Marysville, OH]. Water and fertilizer was delivered at the base of each plant, under the leaf canopy, to minimize the chance of splashing between plots. This helps reduce the potential of transferring bacteria and herbicide between pots and plants.

Plants were inoculated when they reached three visible collars. The two youngest, fully expanded leaves of each plant were inoculated by cutting off the leaf tip and dipping the remaining leaf into the inoculum for 5 s. Non-inoculated plants were injured using the same method, but were not exposed to inoculum. At 7 d after inoculation (DAI), inoculated leaves were visually assessed for percent symptomatic leaf area compared to the whole leaf to confirm successful inoculation and measure disease severity. At time of herbicide application, the average height of annual ryegrass was 13 cm, with an average of 6 leaf collars and 2 tillers, with approximately 5 percent symptomatic tissue from *C. nebraskensis* infection on the inoculated leaves. Giant foxtail averaged 13 cm for height and had 4 leaf collars with approximately 10 percent symptomatic tissue. Large crabgrass was 16.5 cm in height, with 6 leaf collars and 3 tillers with approximately 5 percent symptomatic tissue. Plants were then treated with the herbicides using a single-nozzle spray booth calibrated to

deliver 140 L ha^{-1} at a pressure of 207 kPa using an XR 8002E spray tip (TeeJet Technologies, Urbandale, IA 50322).

3.3.4 Experimental Design

3.3.4.1 Experimental Design for Study 1

Plants were arranged in a randomized complete block design with five replications and repeated twice. In each replication, there were 10 plants per species: 8 were inoculated with *C. nebraskensis*, and 2 served as non-inoculated controls. Seven of the inoculated plants were treated separately with the herbicides listed in Table 3.1, while the last inoculated plant was not treated with any herbicide to serve as a control. Plants were grown in the greenhouse with day/night temperatures of 29/22 C. Due to heating failures in the greenhouse during the second experiment that allowed temperatures to drop as low as 5 C, the experiment was repeated an additional time (for a total of three experiments). Supplemental lighting was provided with 400 watt high-pressure sodium lamps to maintain a 12-h photoperiod.

Visual control ratings were taken 14 d after herbicide treatment (DAT) using a scale of 0 (no control) to 100 (complete plant death). At 14 DAT, inoculated leaves were sampled for laboratory testing to determine if pathogenic *C. nebraskensis* could be recovered from the leaf tissue. One leaf from each plant was examined microscopically (100X) for bacterial streaming. We attempted to sample the edge of symptomatic tissue for streaming, though on many samples it was difficult to differentiate necrotic tissue caused by *C. nebraskensis* compared to necrotic tissue caused by herbicidal activity. Despite that challenge, bacterial streaming was confirmed in over 70 percent of all plants sampled, with no difference between herbicide treatment (*data not shown*).

All inoculated leaves were rinsed with sterile ddH_2O then cultured onto the semi-selective *Corynebacterium nebraskense* selective (CNS) medium minus LiCl (Smidt and Vidaver 1986) to isolate any colonizing bacteria. After 7 d, bacteria were single-colony streaked onto NBY to determine bacteria morphology. Single colonies that matched the morphology of *C. nebraskensis* on NBY were then selected and cultured on new NBY plates for 3 d at 24 C. After 3 d, inoculum was prepared using the same method as section 3.3.2. Inoculum was pooled across weed species source, but separated by herbicide treatment, resulting in eight different sources of inoculum.

Inoculum was then used to inoculate 10 corn plants per herbicide treatment. The corn was a *C. nebraskensis*-susceptible hybrid (DKC55-09RIB Brand Blend, Dekalb® Corn, Monsanto Company, St. Louis, MO) that was direct-seeded into 655-cm^3 cone-tainers (Deepot, Stuewe & Sons, Tangent, OR) filled with potting soil (Redi-Mix, Sun-Gro Redi-Earth Plug and Seedling Mix, Sun-Gro Horticulture, Bellevue, WA). Corn plants were inoculated using the same protocol listed in section 3.3.3. Seven DAI, corn plants were examined for typical symptoms of Goss's wilt to determine if the recovered *C. nebraskensis* remained pathogenic on corn after recovery from herbicide-treated weeds.

3.3.4.2 Experimental Design for Study 2

Plants were arranged in a randomized complete block design with ten replications and repeated once. In each replication, there were 16 plants per species: 8 were inoculated with *C. nebraskensis*, and 8 were injured, but not inoculated. One inoculated plant and 1 non-inoculated plant per species were treated with the herbicides listed in Table 3.1, while the remaining inoculated and non-inoculated plants were not treated with any herbicide to serve as controls. Plants were grown in the greenhouse with day/night temperatures of

30/25 C. Supplemental lighting was provided with 600 watt high-pressure sodium lamps to maintain a 16-h photoperiod.

Visual control ratings were taken at 7, 14, and 21 DAT using a scale of 0 (no control) to 100 (complete plant death). At 21 DAT, above-ground plant tissue was harvested and dried in a forced air dryer and 45 C until constant weight to determine dry weights.

3.3.5 Data Analysis

3.3.5.1 Statistical Analysis for Study 1

Visual ratings for weed control (percent) were checked for normality and homogeneity of variance using PROC UNIVARIATE in SAS 9.4 (SAS Institute, Inc., Cary, NC 27513). Data were non-normal and were Arcsine transformed. Data were back-transformed for presentation. Transformed data were subjected to ANOVA using the PROC GLIMMIX procedure in SAS 9.4. There were no interactions between herbicide treatment and experiments, so data were pooled for analysis. Means were separated using Tukey's Honestly Significant Difference (HSD) test at $P \leq 0.05$.

Percentage of inoculated corn plants that exhibited symptoms of Goss's wilt were subject to Chi-Square analysis using PROC FREQ in SAS 9.4. The data were analyzed using herbicide treatment by number of inoculated plants expressing symptoms of Goss's wilt. There were no interactions between herbicide treatment and experiments, so data were pooled for analysis.

3.3.5.2 Statistical Analysis for Study 2

Visual ratings and dry weights were checked for normality and homogeneity of variance using PROC UNIVARIATE in SAS 9.4. Visual ratings were Arcsine transformed, and dry weights were Log_{10} transformed. Both were back-transformed for presentation.

Transformed data were subjected to ANOVA using PROC GLIMMIX procedure in SAS 9.4. There were no interactions between herbicide treatment and experiments, so data were pooled for analysis. Means were separated using Tukey's HSD test at $P \leq 0.05$.

3.4 Results and Discussion

3.4.1 Weed Control, Pathogen Recovery, and Pathogenicity in Study 1

Clethodim and glyphosate provided the greatest control for all three plant species tested (Table 3.2). Dicamba and the no herbicide control both provided no control for all weeds tested. Atrazine, glufosinate, nicosulfuron, and topramezone provided varying control levels with some differences between the three species. Atrazine and nicosulfuron were the second-best treatments for annual ryegrass control, providing 57 and 65 percent control, respectively, followed by glufosinate with 40 percent control. Topramezone was no different from dicamba or the no herbicide control for annual ryegrass control. Glufosinate and topramezone were the second-best treatments for giant foxtail control, providing 58 and 51 percent control, respectively, followed by nicosulfuron with 18 percent control. Atrazine was no different from dicamba or the no herbicide control for giant foxtail. Topramezone provided the second best weed control for large crabgrass (55 percent), followed by glufosinate and nicosulfuron at 40 percent and 36 percent, respectively. Similar to giant foxtail, atrazine was not different from dicamba and the no herbicide control on large crabgrass. The lower and variable control for atrazine, glufosinate, nicosulfuron, and topramezone across all three plants can be explained due to plants being larger than recommended herbicide label heights and growth stage for control, and inherent weaknesses of those herbicides for controlling these plants (Anonymous 2018a, 2018b, 2018c, 2018d; Loux et al. 2018).

Despite having treatment differences between experiments that led to variable plant kill, there were no differences in pathogen recovery (*data not shown*). In experiment one, plant samples were isolated depending on if they were alive or completely dead at the time of sampling. Bacteria recovered from completely dead plants were kept separate and used to inoculate separate corn plants than *C. nebraskensis* recovered from live plants. There were no differences in recovery or pathogenicity of recovered *C. nebraskensis* in experiment one (*data not shown*), so bacteria were combined across all plant samples, within herbicide treatment, for experiments two and three. Across all three experiments, *C. nebraskensis* was recovered from over 95 percent of sampled plants (*data not shown*). *C. nebraskensis* recovered from herbicide-treated weeds caused Goss's wilt on 93 to 100 percent of inoculated corn samples, with no differences between herbicide treatments (Table 3.3).

3.4.2 Control of Inoculated vs. Non-Inoculated Weeds

The herbicides that provided the greatest annual ryegrass control were clethodim, glyphosate, and nicosulfuron (Table 3.4). Atrazine provided the second best control, while glufosinate and topramezone provided the least control except for dicamba and the no herbicide treatment. As with the first study, these variations in annual ryegrass control can be attributed to natural strengths and weaknesses of each herbicide against this species (Loux et al. 2018). There were no differences between inoculated and non-inoculated annual ryegrass plants within herbicides. This suggests that herbicide performance is not affected when annual ryegrass plants are infected with *C. nebraskensis*.

Similar to annual ryegrass, there were no differences in control of large crabgrass between infected and non-infected plants within herbicides. The only differences observed

among between herbicides, with clethodim and glyphosate providing the most control, followed by topramezone and nicosulfuron. Glufosinate only provided 20 to 27 percent control, while atrazine was not different from dicamba or the no herbicide treatment for large crabgrass control.

Giant foxtail control with glufosinate was reduced when the plants were infected with *C. nebraskensis* compared to non-inoculated plants (35 vs 76 percent; Table 3.4). This difference in control was reflected in the dry weights for infected vs non-infected giant foxtail treated with glufosinate. There were no differences in giant foxtail control with any other herbicides between *C. nebraskensis*-infected and non-inoculated plants. The reduction in control (and subsequently greater biomass) with glufosinate suggests that infection by *C. nebraskensis* is reducing the amount of glufosinate reaching the target site, which hinders herbicide performance in giant foxtail. This could be attributed to a decrease in glufosinate absorption where the *C. nebraskensis* symptomatic tissue was present at herbicide application, or possibly due to reduced translocation of glufosinate due to colonization of the vascular tissue by *C. nebraskensis*. Pline et al. (1999) found that 54 percent of applied glufosinate was absorbed by giant foxtail, which was a relatively high amount amongst weed species. In our study, 10% of the inoculated leaf tissue was symptomatic at herbicide application, resulting in less living tissue available for glufosinate to be absorbed into. Glufosinate is also more mobile in the foxtail (*Setaria*) species than other weeds. Mersey et al. (1990) reported that green foxtail translocated twice the amount of glufosinate out of a treated leaf than barley (*Hordeum vulgare* L.). Pline et al. (1999) reported that as much as 80 percent of absorbed glufosinate will translocate out of giant foxtail leaves, with the majority moving acropetally to the upper foliage, but they could

not differentiate the amount of xylem vs phloem mobility. Shelp et al. (1992) reported that glufosinate is more mobile in the phloem than xylem of soybeans, though it is mobile in both the xylem and phloem. Reduced xylem mobility of herbicides is a possible issue in plants infected with *C. nebraskensis* since the bacteria mostly colonize xylem vascular tissue. Mbofung et al. (2016) found that *C. nebraskensis* was restricted to growth in the xylem in resistant corn hybrids, whereas in susceptible hybrids the xylem was indistinguishable from nearby cells due to lysing of the cells. Campbell (2017) reported that symptomology of giant foxtail is more similar to a resistant corn hybrid than a susceptible corn hybrid when exposed to higher inoculum levels. This might suggest that *C. nebraskensis* is similarly restricted to xylem tissue in giant foxtail like in a resistant corn hybrid.

In our experiment, symptoms of *C. nebraskensis* infection were not observed on any leaves except the inoculated leaves in any weed species (*Data not shown*). This makes it more likely that reduced absorption has a greater effect on herbicide efficacy than reduced xylem translocation since there was no visual evidence of *C. nebraskensis* colonizing the non-inoculated leaves, which make up the majority of the leaves on the plants. If colonization only occurred in the inoculated leaves, then only the xylem of two leaves per plant would have been blocked and led to reduced herbicide translocation.

One other potential cause of reduced efficacy of glufosinate on *C. nebraskensis*-infected giant foxtail is the metabolism of small amounts of glufosinate by the bacteria. Pline et al. (2001) reported that *Pseudomonas syringae* can metabolize glufosinate when exposed to very low (1 mM) amounts of the herbicide. However, once exposed to higher rates (100 mM), glufosinate antagonized *P. syringae* growth, suggesting antimicrobial

activity. While these experiments have not been tested on any *Clavibacter* species, it remains possible that reduced absorption on inoculated leaves exposed the *C. nebraskensis* inside giant foxtail to low rates of glufosinate, and potential metabolism occurred.

3.5 Conclusions

In our study, we found that *C. nebraskensis* can be recovered from herbicide-treated weeds and remain pathogenic after recovery. This is important for disease management because it highlights that once alternative hosts have become infected with *C. nebraskensis*, there are no effective chemical methods to decrease pathogenicity of the bacteria that have colonized those hosts. The no-herbicide treatment also shows that killing weeds with mechanical methods will also not reduce pathogenicity of *C. nebraskensis*. While the list of herbicides evaluated could have been more comprehensive, and more focused on herbicides that provide excellent control of the targeted weed species, we evaluated active ingredients that will be widely used across the Midwest within 7 unique modes of action. This allowed us to evaluate herbicides that target different mechanisms within a plant that could also potentially target a mechanism with *C. nebraskensis*.

Controlling alternative hosts is still important so that they are killed when small, which limits the amount of time that *C. nebraskensis* has to colonize and reproduce within the plants and subsequently increase inoculum levels in a field. This reinforces the importance of controlling weeds when they are small as it can potentially limit the increase of inoculum levels in a field, and also inhibits alternative hosts acting as a "bridge host" for *C. nebraskensis* in years when a field is rotated to a crop besides corn. Our findings also support the practice of applying residual herbicides. In addition to providing better weed control and preventing weed competition to crops, the use of residual herbicides can

also prevent alternative hosts from actively growing and becoming potential reservoirs for *C. nebraskensis*.

The fact that glufosinate has reduced efficacy on giant foxtail that is infected with *C. nebraskensis* is another reason to support the use of preemergence herbicides for weed control. We would expect a similar decrease in efficacy of glufosinate with the other *Setaria* species due to their similarity with giant foxtail. Further research is necessary to determine the exact mechanism that led to decreased efficacy of glufosinate on *C. nebraskensis*-infected giant foxtail. Regardless of the mechanism that leads to decreased control of *C. nebraskensis*-infected giant foxtail with glufosinate, our results show that glyphosate and clethodim can be good tank-mix partners for grass weed control since their efficacy was not affected by the presence of *C. nebraskensis* in giant foxtail (Table 3.4). Until the mechanism is elucidated, glufosinate should not be used as the sole method of grass weed control in commercial corn and soybean fields with a history of Goss's wilt.

3.6 Acknowledgments

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Table 3.1. Sources of commercial herbicides used in greenhouse experiments

Common name		Manufacturer	Mode of action	Rate	Adjuvants used[a]
				g ha^{-1}	
Atrazine	Aatrex® 4L	Syngenta Crop Protection, LLC	Photosystem II inhibitor	1680	COC
Clethodim	Select Max®	Valent USA Corp.	ACCase inhibitor	136	AMS + COC
Dicamba	Clarity®	BASF Corp.	TIR1 auxin receptor	560	AMS
Glufosinate	Liberty® 280 SL	Bayer CropScience, LP	Glutamine synthase inhibitor	593	AMS
Glyphosate	Roundup® Powermax	Monsanto Co	EPSPS inhibitor	1260	AMS
Nicosulfuron	Accent® Q	DuPont	ALS inhibitor	35	AMS + COC
Topramezone	Armezon™	BASF Corp.	HPPD inhibitor	18.4	AMS + MSO

[a]Abbreviations: AMS = ammonium sulfate; COC = crop oil concentrate; MSO = methylated seed oil.

Table 3.2. Control of *Clavibacter nebraskensis*-infected annual ryegrass, giant foxtail, and large crabgrass at 14 days after herbicide treatment in Study 1.

Herbicide	Rate	Annual ryegrass[a]	Giant foxtail	Large crabgrass
	g ha^{-1}	----------%----------		
Atrazine	1680	57 b	3 d	4 d
Clethodim	136	97 a	92 a	96 a
Dicamba	560	0 d	0 d	0 d
Glufosinate	593	40 c	58 b	40 c
Glyphosate	1260	93 a	100 a	82 a
Nicosulfuron	35	65 b	18 c	36 c
Topramezone	18.4	5 d	51 b	55 b
No herbicide	0	0 d	0 d	0 d

[a]Mean values separated using Tukey's HSD. Values followed by different letters within column are significantly different ($P \leq 0.05$).

Table 3.3. Percentage of corn plants exhibiting typical symptoms of Goss's wilt after inoculation with *Clavibacter nebraskensis* recovered from infected, herbicide-treated weeds.

Herbicide	Rate	Corn[a,b]
	g ha^{-1}	---------%---------
Atrazine	1680	93 NS
Clethodim	136	100 NS
Dicamba	560	100 NS
Glufosinate	593	93 NS
Glyphosate	1260	97 NS
Nicosulfuron	35	93 NS
Topramezone	18.4	100 NS
No herbicide	0	100 NS

[a]Data are pooled within herbicides, across weed species (annual ryegrass, giant foxtail, and large crabgrass) and experiments. Tukey's HSD (0.05) = not significant (NS).
[b]Column represents the percentage of inoculated plants expressing typical symptoms of Goss's wilt out of all inoculated corn plants.

Table 3.4. Control and dry weight biomass of *Clavibacter nebraskensis*-infected and non-infected annual ryegrass, giant foxtail, and large crabgrass at 21 days after herbicide treatment in Study 2.

Herbicide	Rate	Inoculated	Annual ryegrass		Giant foxtail		Large crabgrass	
	g ha^{-1}		% control[a]	Dry weight	% control	Dry weight	% control	Dry weight
Atrazine	1680	Yes	73 b	0.12 d	44 e	0.34 cd	5 e	1.53 abc
		No	65 b	0.17 cd	44 e	0.41 cd	6 e	1.38 abcd
Clethodim	136	Yes	100 a	0.16 cd	100 a	0.16 def	99 a	0.50 e
		No	100 a	0.16 c	100 a	0.12 ef	100 a	0.52 e
Dicamba	560	Yes	0 d	0.53 a	0 f	1.48 a	0 e	1.85 ab
		No	0 d	0.54 a	0 f	1.14 ab	0 e	2.00 a
Glufosinate	593	Yes	17 c	0.34 b	35 e	0.65 bc	20 d	1.46 bcd
		No	17 c	0.35 ab	76 d	0.26 de	27 d	1.49 abcd
Glyphosate	1260	Yes	98 a	0.10 d	100 a	0.14 ef	100 a	0.25 f
		No	100 a	0.15 cd	100 a	0.10 f	100 a	0.23 f
Nicosulfuron	35	Yes	94 a	0.13 cd	84 cd	0.21 de	71c	1.00 cd
		No	96 a	0.17 c	88 bcd	0.17 def	81 bc	0.84 d
Topramezone	18.4	Yes	17 c	0.35 ab	91 bc	0.19 de	83 bc	0.50 e
		No	12 cd	0.30 b	94 b	0.14 ef	87 b	0.40 e
No herbicide	0	Yes	0 d	0.48 ab	0 f	1.56 a	0 e	1.56 abc
		No	0 d	0.55 a	0 f	1.44 a	0 e	1.84 ab

[a] Mean values separated using Tukey's HSD. Values followed by different letters within column are significantly different ($P \leq 0.05$)

CHAPTER 4. ABILITY OF *CLAVIBACTER NEBRASKENSIS*, CAUSAL AGENT OF GOSS'S WILT OF CORN, TO OVERWINTER IN ALTERNATIVE HOST DEBRIS AND SEED

4.1 Abstract

Goss's bacterial wilt and leaf blight of corn is caused by the Gram positive bacterium *Clavibacter nebraskensis*. Goss's wilt has been identified in 16 states in the United States (US) and has become an increasingly important disease over the last decade. The cause of the recent re-emergence and spread of the disease is unknown, but has been attributed to an increase in hectares planted corn-on-corn, an increase in no-tillage practices, and wide-spread use of corn hybrids that are susceptible to *C. nebraskensis*. Some grass weed species and cover crops have been documented as alternative hosts of *C. nebraskensis*, although their role as an additional source of inoculum has not been researched. To answer this question, two studies were initiated to investigate the potential of *C. nebraskensis* to overwinter on alternative hosts. In the first study, giant foxtail, large crabgrass, and a *C. nebrakensis*-susceptible corn hybrid were inoculated with *C. nebraskensis*, and plants with confirmed symptoms were buried in December 2013 in a field at the Agronomy Center for Research and Education near West Lafayette, Indiana, at 0 and 15 cm below the surface. Plant debris was sampled every four months for two years. Results from this study reveal that *C. nebraskensis* can overwinter and remain pathogenic on both corn and alternative host debris in Indiana for up to 4 months, but no pathogenic *C. nebraskensis* was recovered at or after the 8 month sampling period. In the second study, annual ryegrass, giant foxtail, and johnsongrass were inoculated in the greenhouse with a bacterial suspension containing 1 x 10^8 colony-forming units (CFU) of *C. nebraskensis* per ml with three different

treatments: inoculation at the three-collar growth stage (V3) + six-collar growth stage (V6) + seed-head emergence, inoculation at V6 + seed-head emergence, and inoculation at seed-head emergence only. Plants were grown to maturity, seed were collected from all plants, and rhizomes were collected from johnsongrass. Seed and rhizomes were tested in the lab for the presence of *C. nebraskensis*, and none was found. Alternative hosts did not become systemically infected, with symptomatic lesions never exceeding 65% on any inoculated leaf. Results from these studies indicate that *C. nebraskensis* can overwinter on infected host debris in Indiana, but the bacterium is not seed-borne in alternative hosts.

4.2 Introduction

Goss's bacterial wilt and leaf blight (Goss's wilt) was first discovered in a hybrid corn (*Zea mays* L.) field in Nebraska in 1969 (Schuster et al. 1972). It is caused by the Gram positive bacterium *Clavibacter nebraskensis* comb. nov. (Li et al. 2018). The disease was widespread in the western corn belt throughout the 1970's, but only sporadic cases were reported from the 1980's until the disease reemerged in western Nebraska, southeast Wyoming, and eastern Colorado in 2006 (Jackson et al. 2007). Reemergence was attributed to an increase in continuous corn cropping practices, an increase in conservation tillage, and widespread planting of *C. nebraskensis*-susceptible corn hybrids (Jackson et al. 2007). The disease has spread throughout the Midwestern US since reemergence, and it is currently known to be in 16 states in the United States (US): Colorado, Illinois, Indiana, Iowa, Kansas, Louisiana, Minnesota, Missouri, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas, Wisconsin, and Wyoming (Friskop et al. 2014; Hosack et al. 2016; Jackson et al. 2007; Korus et al. 2011; Malvick et al. 2010; Ruhl et al. 2009; Schuster 1975; Singh et al. 2015; Wysong et al. 1973; Yasuhara-Bell et al. 2016). Goss's

wilt was the third-leading cause of corn yield loss due to disease in the Midwestern US and Ontario, Canada between 2012 and 2015, when it caused an estimated loss of 13 million metric tons of corn (Mueller et al. 2016). Yield in individual fields can exceed 50 percent, though losses only up to 44 percent have been documented in research trials (Carson and Wicks III 1991; Jardine and Claflin 2016).

Schuster (1975) documented that *C. nebraskensis* overwinters on corn debris, the corn seed-coat, and within corn seed. Eggenberger et al. (2016) confirmed that infected corn residue is a primary source of inoculum. Infected kernels allow for long-distance transportation, but only demonstrate seed-to-seedling transmission at rates below 1 percent (Biddle et al. 1990). There are several known alternative hosts to *C. nebraskensis,* though the role of alternative hosts as an inoculum source is unknown. Commonly found weedy hosts of *C. nebraskensis* include bristly foxtail [*Setaria verticillata* (L.) Beauv.], giant foxtail (*Setaria faberi* Herm.), green foxtail [*Setaria viridis* (L.) Beauv.], yellow foxtail [*Setaria pumila* (Poir.) Roemer & J.A. Schultes], large crabgrass [*Digitaria sanguinalis* (L.)], shattercane [*Sorghum bicolor* (L.) Moench ssp. *Arundinaceum* (Desv.) de Wet & Harlan], johnsongrass [*Sorghum halepense* (L.) Pers.], and woolly cupgrass [*Eriochloa villosa (Thunb.) Kunth]* (Langemeier et al. 2014; Ikley et al. 2015, Webster 2016). Ikley et al. (2015) found the commonly used cover crop annual ryegrass (*Lolium multiflorum* Lam.) is also an alternative host. A survey by Campbell (2017) found that giant foxtail, yellow foxtail, and large crabgrass were found in 94, 74, and 63 percent of fields, respectively, in regions of Indiana that have fields with a history of Goss's wilt. Giant foxtail and large crabgrass have also been documented as common weed escapes in soybean fields across Indiana (Johnson et al. 2004). The prevalence of alternative hosts of *C. nebraskensis* in

these fields is concerning due to their potential to increase inoculum within a growing season, and as an additional source of inoculum for future years.

In all published research, *C. nebraskensis* has been isolated from alternative host leaf tissues that were sampled while still alive and actively growing in either the greenhouse or field. It is unknown if *C. nebraskensis* can overwinter on alternative host debris or within reproductive structures. There is limited information about bacterial diseases of corn surviving and overwintering on alternative hosts (Munkvold and White 2016). Previous research has shown that *Pseudomonas syringae*, the bacterial causal agent of brown spot on soybean, can overwinter on alternative hosts (Ercolani et al. 1974). More research is published on the ability of viral and fungal diseases of corn to overwinter on or in alternative hosts. Johnsongrass is a main overwintering host of the corn diseases Maize Dwarf Mosaic Virus and Maize Chlorotic Dwarf Virus. Both viruses are capable of surviving in johnsongrass rhizomes and infecting new above-ground growth in subsequent years (Stewart et al. 2016). These viruses require an insect vector to transmit the pathogen from johnsongrass to corn (Eberwine and Hagood 1995). The fungus *Fusarium graminearum* has been widely researched due to its status as an important pathogen of both corn and wheat (*Triticum aestivum* L.). In addition to wheat, other small grains have been confirmed as alternative hosts and additional inoculum reservoirs for the fungus (Sutton 1981). Due to the limited research on the ability of bacterial diseases of corn, and specifically *C. nebraskensis*, to overwinter on or inside alternative hosts, we initiated two studies to address this topic. The objective for study one was to determine if *C. nebraskensis* can overwinter and remain pathogenic on giant foxtail, large crabgrass, and corn debris in Indiana. The objectives of study two were to (1) determine if *C. nebraskensis*

can be transmitted to the seed of three different alternative hosts, and (2) determine if *C. nebraskensis* can colonize the rhizomes of johnsongrass.

4.3 Materials and Methods

4.3.1 Burial Study

4.3.1.1 Species Selection and Sample Preparation

Giant foxtail and large crabgrass were chosen as weedy alternative hosts to test survival of *C. nebraskensis* on due to their prevalence in many Indiana fields. They are common grass weed escapes in soybean fields, and are also found in 94 and 63 percent of fields with a history of Goss's wilt (Campbell 2017; Johnson et al. 2004). A *C. nebraskensis*-susceptible corn hybrid (DKC55-09RIB Brand Blend, Dekalb® Corn, Monsanto Company, St. Louis, MO) was also included for comparison, and to determine overwintering capabilities of *C. nebraskensis* on corn in Indiana since that has not previously been tested.

Giant foxtail and large crabgrass were grown and inoculated in the greenhouse in order to prepare samples for burial. Plants were direct-seeded into pots, measuring 15.25 cm in diameter by 17.75 cm in depth, filled with a 50:50 mix of sand:potting soil (Redi-Mix, Sun-Gro Redi-Earth Plug and Seedling Mix, Sun-Gro Horticulture, Bellevue, WA). Every leaf of the plants were inoculated with a bacterial suspension containing 1×10^8 colony-forming units (CFU) of *C. nebraskensis* per ml at the six-collar growth stage by cutting off the leaf tip and dipping the remaining leaf into the inoculum for 5 s. At 14 d after inoculation, plants were harvested by clipping the stem at the soil surface, and above-ground tissue were dried in the greenhouse. Corn plants were grown in the field in 2013 and inoculated with a bacterial suspension containing 1×10^6 CFU of *C. nebraskensis* per ml at the six-collar leaf stage (V6) with a modified pin-prick device (Figure 4.1). Plants

confirmed to have typical leaf blight symptoms of Goss's wilt were harvested at plant maturity and brought into the greenhouse to dry for burial preparation. Corn plants were dissected so that one node and its leaf, plus 10 cm of stem below and above the node were samples to be buried.

After drying, two whole plants of giant foxtail and large crabgrass, plus two nodes of corn were placed into individual 30.5 x 15.25-cm charcoal fiberglass mesh bags (New York Wire, Hanover, PA), which were the placed into 61 x 30.5-cm bags made from aluminum mesh screen (New York Wire, Hanover, PA). This allowed plants to be recovered easily without interfering with soil to surface area contact of plant tissue.

4.3.1.2 Field Description and Experimental Design.

The field experiment was established in December 2013 at the Agronomy Center for Research and Education (ACRE) near West Lafayette, IN. The experimental design was a split-plot with four replications, where the whole plot was burial depth (0 and 15 cm), and subplots were sampling times of 0, 4, 8, 12, 16, 20, and 24 months after burial. Average temperature and monthly precipitation throughout the experiment are listed in Table 4.1. Treatments were replicated four times, and each replication contained a plant sample for each sampling time and burial depth. Bags were laid flat to ensure equal soil exposure to plant surfaces. A soil depth of 15 cm ensured the buried plants did not move, and plants at 0 cm were anchored with pins measuring 15 x 2.5 x 15 cm (Dewitt, Sikeston, MO). Plots were established in separate parts of the field, representing the field edge, headland row, and two replications in the field interior. The replications in the field interior were placed on two separate soil types: a Drummer silty clay loam, and a Raub-Brenton silt loam (Figure 4.2).

4.3.1.3 Sampling Procedure and Pathogenicity Testing

One bag of each species at each burial depth per replication were recovered at each sampling timing. Corn samples were separated into stem and leaf tissue for testing. Starting at the 4 month sampling time, giant foxtail and large crabgrass stem and leaf tissue could not be differentiated, so they were tested as whole plants. Samples were surface sterilized for 1 minute in 10-percent NaOCL, then rinsed in sterilized double-distilled water (ddH_2O) for 5 minutes. Samples were then macerated in 50 ml sterilized ddH_2O. After 4 hours, 100 µl aliquots of 10-fold serial dilutions down to 1:1000 of the supernatant were plated onto the *Corynebacterium nebraskense* selective (CNS) medium minus LiCl (Smidt and Vidaver 1986a). Bacteria were allowed to grow on plates for up to 14 d at 24 C. Due to limited bacterial growth on CNS, individual colonies resembling the morphology of *C. nebraskensis* were single-colony streaked onto Nutrient Broth Yeast (NBY) to confirm bacteria morphology. Bacterial colonies that matched the description of *C. nebraskensis* isolated on NBY (apricot-orange in color, circular, convex, glistening, and butyrous delimited from a darker center zone; Vidaver and Mandel 1974) were selected for pathogenicity testing.

Selected bacteria were cultured on Nutrient Broth Yeast (NBY) media for 3 d at 24 C. Bacterial colonies were suspended in sterilized ddH_2O. Inoculum was adjusted to a concentration of 1×10^8 CFU per ml with a Spectrophotometer (Beckman Coulter DU 530, Beckman Coulter, Inc., Brea, CA) set at 600 nanometers (nm). Inoculum was kept separate by species and burial depth that the bacteria were isolated from, and was used to inoculate ten *C. nebraskensis*-susceptible corn plants per treatment at the three-collar (V3) growth stage in the greenhouse. Plants were inoculated by cutting off the leaf tip and dipping the

remaining leaf into the inoculum for 5 s. Corn plants were then examined at 7 and 14 days after inoculation for typical symptoms of Goss's wilt.

Starting at the 8-month after burial sample timing, the sampling procedure was slightly modified due to contamination from other bacteria on CNS that made it difficult to determine morphology of *C. nebraskensis* from other apricot-orange bacteria. Gross and Vidaver (1979) reported than some coryneform and coccoid soil bacteria can also grow on CNS, so this could have been a source of bacterial contamination we found in this study. The sampling procedure remained the same as earlier sampling times until the step where supernatant was plated onto CNS. For the modified sampling procedure, the *C. nebraskensis*-susceptible corn hybrid was used as the recovery medium. Plants were directly inoculated with the supernatant by both cutting the leaf tip and dipping the remaining leaf into the inoculum for 5 s, and by injecting 10 ml of supernatant directly into the stem 2.5 cm above the soil line. Using corn plants as the recovery medium is the same method Schuster (1975) used in the only published study evaluating survival of *C. nebraskensis* in plant debris. Supernatant was also tested using Agdia Immunostrips® test kits (Agdia Inc., Elkart, IN) that are designed for detection of *C. michaganensis*, but have been confirmed to be effective in testing for *C. nebraskensis* (Korus 2011). Corn plants were then rated at 7 and 14 d after inoculation for typical symptoms of Goss's wilt.

4.3.2 Seed Transmission Study

4.3.2.1 Species Selection and Inoculum Preparation

Annual ryegrass, giant foxtail, and johnsongrass were chosen as species to test in this study since they are alternative hosts of *C. nebraskensis* that have different plant life cycles (winter annual, summer annual, and perennial, respectively). Annual ryegrass is the only

known winter annual host of *C. nebraskensis*, and johnsongrass is the only known perennial that is classified as a weed, and not a native grass (Ikley et al. 2015; Langemeier et al. 2014; Schuster 1975; Webster 2016). Of the known summer annual hosts, giant foxtail was selected due to its prevalence in fields with a history of Goss's wilt across the Corn Belt (Campbell 2017; Langemeier et al. 2017).

For inoculation, a confirmed strain of *C. nebraskensis* that was isolated from Pulaski county Indiana in 2008 and stored in a 20 percent glycerol solution at minus 80 C was obtained from the Purdue Plant and Pest Diagnostic Laboratory (Ruhl et al. 2009). The isolate was grown on Nutrient Broth Yeast (NBY) media for 3 d at 24 C. Bacterial colonies were suspended in sterile ddH_2O and inoculum concentration was adjusted to a concentration of 1 x 10^8 CFU per ml with a Spectrophotometer set at 600 nm.

4.3.2.2 Inoculation Method and Timing

Annual ryegrass, giant foxtail, and johnsongrass were all directly sown into pots measuring 15.25 cm in diameter by 17.75 cm in depth, filled with a 50:50 mix of sand:potting soil (Redi-Mix, Sun-Gro Redi-Earth Plug and Seedling Mix, Sun-Gro Horticulture, Bellevue, WA). Plants were watered daily and fertilized weekly [Miracle-Gro® Water Soluble All Purpose Plant Food (24-8-16); Scotts Miracle-Gro Products Inc., Marysville, OH]. Water and fertilizer were delivered at the base of each plant, under the leaf canopy, to minimize the chance of splashing bacteria between pots.

Plants were arranged in a randomized complete block design with three replications, and the experiment was repeated once. Each replication contained five plants per species per treatment. Treatments consisted of four different inoculation timings: inoculation at (1) three-collar plus six-collar plus seedhead emergence growth stages, (2) six-collar plus

seedhead emergence growth stages, (3) seedhead emergence only growth stage, and (4) a non-inoculated control. This treatment structure was chosen because it was used by Biddle et al. (1990) in their study to determine seed transmission of *C. nebraskensis* in corn. Plants were grown in the greenhouse with day/night temperatures of 30/25 C. Supplemental lighting was provided with 600 watt high-pressure sodium lamps to maintain a 16-h photoperiod.

At each inoculation timing, every full leaf present was inoculated using a modified pin-prick device (Figure 4.3). This device allowed for 5 wounds to be made on small plant leaves while simultaneously delivering approximately 5 ml of inoculum per plant. The sponge end of the device was dipped into the inoculum between each plant. Plants were evaluated for visual symptoms of *C. nebraskensis* infection at 14 days after each treatment to confirm successful inoculation and measure disease severity on a scale of 0 to 100 (with 0 representing no symptoms and 100 representing complete leaf death). Disease severity was recorded by averaging the percent of symptomatic tissue over all inoculated leaves on a plant. Plants were also observed for spread of any symptoms beyond the inoculated leaves, which would indicate systemic infection by *C. nebraskensis*. Plants were allowed to grow until maturity, then seed was collected from all three species, and rhizomes were sampled from johnsongrass.

4.3.2.3 Seed Sampling Procedure

Seed were pooled across the 5 plants per plot, but kept separate by species and replicate. The seed were surface sterilized for 1 minute in 10-percent NaOCL, then rinsed in sterilized ddH_2O for 5 minutes. Seed were then allowed to dry overnight in a sterile laminar-flow hood. After drying, Twenty-five seed from each plot were directly plated onto CNS, and

twenty-five seed from each plot were directly plated onto NBY, with each media replicated twice. This resulted in 100 seed per species per plot being directly plated onto recovery media. An additional 1,000 seed per species per plot were ground in a Thomas Model 4 Wiley® Mill (Thomas Scientific, Swedesboro, NJ). Thirty ml of sterilized ddH_2O was added to the ground seed and allowed to soak for 4 hours. 100 µl aliquots of 10-fold serial dilutions down to 1:1000 of supernatant were plated onto both CNS and NBY. Plates were incubated for 7 to 14 d at 22 C to observe bacterial growth. Any bacteria resembling *C. nebraskensis* were single-colony streaked onto NBY and allowed to grow for 3 d at 22 C. Single colonies were then subjected to testing with Agdia Immunostrips® test kits, and used to inoculate a *C. nebraskensis*-susceptible corn hybrid in the greenhouse. Single colonies were cultured on NBY media for 3 d at 22 C, suspended in sterile ddH_2O and inoculum concentration was adjusted to 1 x 10^8 CFU per ml Corn plants were inoculated by cutting off the leaf tip and dipping the remaining leaf into the inoculum for 5 s. Corn was rated 7 and 14 d after inoculation for typical symptoms of Goss's wilt. Twenty-five g of johnsongrass rhizomes per plot were tested following the same procedures listed for the ground seed samples.

4.3.3 Statistical Analysis

Visual ratings for percent disease severity of giant foxtail were checked for normality and homogeneity of variance using PROC UNIVARIATE in SAS 9.4 (SAS Institute, Inc., Cary, NC). The non-inoculated check was not included in data analysis. Data were non-normal and were Arcsine-square-root transformed to meet normality assumptions. Transformed data were subjected to ANOVA using the PROC GLIMIX procedure in SAS 9.4, and data were back-transformed for presentation. There was an interaction between treatments and

experiments, so experiment data are presented separately. There was no difference in disease severity between treatments (number of inoculations) within inoculation timing in either experiment (*data not shown*), so treatments were combined for analysis to test differences in disease severity between inoculation timings. Means were separated using Tukey's Honestly Significant Difference (HSD) test at $P \leq 0.05$. Due to low disease severity in both experiments, annual ryegrass and johnsongrass were not subjected to analysis.

4.4 Results and Discussion

4.4.1 Burial Study

At the time of burial, plant tissue was tested to determine the baseline number of CFU per g of tissue. Corn stem tissue averaged 3.4×10^5 CFU per g and corn leaf tissue averaged 6.2×10^5 CFU per g. Symptoms of *C. nebraskensis* infection were only noted on giant foxtail and large crabgrass leaf tissue, and *C. nebraskensis* was only recovered on media from leaf tissue. Giant foxtail leaves averaged 6.2×10^4 CFU per g, and large crabgrass leaves averaged 2.8×10^3 CFU per g. Our goal was to determine the decrease in CFU per g of plant tissue over time, but due to an abundance of other bacteria, it was not possible to differentiate and count colonies of *C. nebraskensis* at any sampling time after 0 months. Smidt and Vidaver (1986b) were able to quantify *C. nebraskensis* populations on corn by sampling living plants during the growing season and plating samples onto CNS. They had difficulty detecting *C. nebraskensis* once populations dropped as low as 1×10^5 CFU per g of fresh corn weight due to other background bacteria. This helps explain why we could detect *C. nebraskensis* at burial time from field grown corn plants since there were more than 1×10^5 CFU per g. We found fewer bacteria in giant foxtail and large crabgrass, but

those plants were grown in the greenhouse which likely helped reduce the number of background bacteria and allowed for initial colony counts.

At 0 and 4 months after burial, we were able to recover bacteria that resembled *C. nebraskensis* morphology on CNS for each plant sample and burial depth. Bacterial colonies resembling *C. nebraskensis* were single-colony streaked onto NBY. Single colonies that matched the morphology of *C. nebraskensis* were cultured on NBY for 3 d and 22 C, then used to inoculate the *C. nebraskensis*-susceptible corn plants in the greenhouse. Putative isolates from corn stems, corn leaves, giant foxtail, and large crabgrass all tested positive using Agdia Immunostrips® and caused typical symptoms of Goss's wilt on 100 percent of corn plants inoculated in the greenhouse (Tables 4.2 and 4.3). This indicates that pathogenic *C. nebraskensis* was able to overwinter on corn, giant foxtail, and large crabgrass and persist into the typical corn planting window in West Lafayette, IN.

At the 8 month sampling time, corn leaves and giant foxtail buried at 15 cm had had completely decomposed and no plant tissue was recovered (Tables 4.2 and 4.3). By the 12 month sampling time, large crabgrass buried at 15 cm had also completely decomposed. By 16 months, corn leaves on the soil surface had fully decomposed, and by the 24 month sampling time, only the corn stem, giant foxtail, and large crabgrass at 0 cm had any plant tissue remaining. This limited the number of samples starting at the 8 month sample timing. For the treatments with no tissue remaining, it is unlikely that pathogenic *C. nebraskensis* remained in the soil since pure colonies have not been able to survive in the soil in previous studies (Schuster 1975).

Starting at the 8 month sampling time, background bacteria became too numerous to use CNS as the recovery medium. When the *C. nebraskensis*-susceptible corn hybrid was used as the recovery medium, inoculum from plant samples at any burial depth did not cause typical symptoms of Goss's wilt for the rest of the sampling times (Table 4.3). Corn plants were observed for symptoms of Goss's wilt for 14 d after inoculation, which is sufficient time to detect inoculum concentrations of 1×10^2 CFU per ml or greater on this corn hybrid in the greenhouse (Campbell 2017). Campbell (2017) confirmed symptoms of Goss's wilt between 15 and 18 d after inoculation for inoculum concentrations of 1×10^1 and 1×10^0 in the greenhouse using the same inoculation method we used in this study. Due to the constraints in which we tested for pathogenicity, it is possible that *C. nebraskensis* was present at very low levels in plant tissue that we were not able to detect since we did not examine plants beyond 14 d after inoculation.

At the 8 month sampling, the Agdia Immunostrips® tested positive for 100 percent of corn leaves buried at 0 cm, 100 percent and 25 percent of corn stems buried at 0 and 15 cm, respectively, and 50 percent of large crabgrass samples at 0 cm. Giant foxtail at 0 cm, and large crabgrass at 15 cm had 0 positive results. These test strips have been proven to consistently detect *C. nebraskensis*, but those tests were performed with bacterial concentrations of 1×10^5 and 1×10^6 CFU per ml (Korus 2011). No data are available testing concentrations of *C. nebraskensis* lower than that, so it remains possible that the Agdia Immunostrips® could have detected very low populations of *C. nebraskensis* in our study. However, the manufacturer also notes that test strips will read positive in the presence of *Xanthomonas campestris* pv. *translucens*, *Microbacterium paraoxydans*, and at least three *Ochrobactrum* species, which are found globally at high concentrations in

soil (Anonymous 2016**,** 2018). It is currently unknown if other bacteria can results in false positive results with these strips. The fact that we did not observe typical symptoms of Goss's wilt, combined with the potential for false positives in the presence of common soil bacteria using the Agdia Immunostrips®, suggests that there was not enough pathogenic *C. nebraskensis* remaining in plants samples at the 8 month through the 24 month sampling time to cause Goss's wilt on corn.

4.4.2 Seed Transmission Study

4.4.2.1 Disease Severity at Different Inoculation Timings

Annual ryegrass and johnsongrass were not subjected to analysis due to low disease severity at all inoculation timings in both experiments. The majority of plants had less than 5 percent, and less than 1 percent symptomatic tissue on inoculated leaves, respectively, across all inoculation timings. Annual ryegrass had 3 plants with leaf symptoms above 5 percent in experiment two, with a maximum of 20 percent (*data not shown*). Johnsongrass had 5 plants with leaf symptoms above 1 percent in experiment one, with a maximum of 20 percent, and 3 plants with leaf symptoms above 1 percent in experiment two, with a maximum of 50 percent (*data not shown*). For both annual ryegrass and johnsongrass, the plants with leaf symptoms above 5 and 1 percent, respectively, occurred after the six-collar inoculation timing. Disease symptoms never spread beyond the hypersensitive response in johnsongrass once it occurred with 48 to 72 hours after inoculation for any inoculation timing. This reaction was previously reported on plants inoculated at the three-collar growth stage, but this is the first report of it continuing to occur through vegetative and into reproductive growth stages (Ikley et al. 2015).

There was a treatment by experiment interaction for disease severity on giant foxtail. In experiment one, there was more disease severity at the three-collar inoculation timing (17 percent), followed by the six collar timing (9 percent), with the least severity observed at the seedhead emergence inoculation timing (1 percent; Table 4.4). In experiment two, the six collar inoculation timing had the most disease severity (10 percent), followed by the three collar timing (6 percent), with the seedhead emergence timing having the least symptoms (4 percent; Table 4.4). Results from experiment one indicate that susceptibility of giant foxtail to *C. nebraskensis* decreases with increasing plant maturity. This trend was previously reported on corn (Calub et al. 1974). Results from experiment two suggest that giant foxtail is more susceptible at the six collar growth stage than at the three collar growth stage, which contradicts the results from experiment one and from previous studies on corn. In both experiments, giant foxtail was more resistant to *C. nebraskensis* infection at seedhead emergence than in vegetative growth stages, which agrees with previous reports in corn of increased resistance once plants enter the reproductive phases compared to vegetative stages. Across both experiments, the maximum percent symptomatic leaf tissue for any individual leaf of giant foxtail was 65 percent, though symptoms did not reach the base of the leaf (*data not shown*). This suggests that systemic infection did not occur in giant foxtail. Giant foxtail has been reported as having similar *C. nebraskensis* susceptibility to corn hybrids rated as moderately-tolerant (Campbell 2017). Mbofung et al. (2016) reported that lesions in resistant corn hybrids would spread towards the leaf tip, but movement towards the base of the leaf was restricted. Lesion development was proceeded by an increase in bacterial colonies in the xylem. Further movement within resistant corn tissue was imperceptible after 16 days after inoculation, with a dense matrix

in the xylem attributed to restricting bacterial movement within the plant. It is possible that giant foxtail has a similar mechanism of tolerance to *C. nebraskensis* since we did not see further spread of symptoms after 14 days after inoculation, and it was reported by Campbell (2017) as having similar disease response as the moderately-tolerant hybrid tested. (*data not shown*).

4.4.2.2 Transmission into Seed and Rhizomes

All plant species tested produced mature seed in experiment one only. In experiment two, annual ryegrass continuously tillered until it eventually died prior to seedhead emergence for all treatments, including the non-inoculated control (*data not shown*). Johnsongrass above-ground leaf tissue senesced prior to producing mature seed, so only the rhizomes were harvested in experiment two. Giant foxtail grew to seed maturity in both experiments.

No bacteria were recovered from whole seed, ground seed, or rhizomes for johnsongrass across both experiments (*data not shown*). Bacteria were not recovered from whole seed samples of giant foxtail in either experiment. Only one species of bacteria was recovered from ground seed of giant foxtail in experiment two. The recovered bacteria did not resemble *C. nebraskensis* morphology on CNS or NBY, tested negative to the Agdia Immunostrips®, and did not cause symptoms of Goss's wilt on inoculated corn (*data not shown*). Bacterial colonies were recovered from whole seed samples of 5 annual ryegrass plots, and ground seed samples of 4 annual ryegrass plots from experiment one. None of the bacteria recovered resembled *C. nebraskensis* morphology on CNS or NBY, all tested negative to the Agdia Immunostrips®, and they did not cause symptoms of Goss's wilt on inoculated corn (*data not shown*).

Results from seed sampling suggest that *C. nebraskensis* cannot be transmitted to seed of annual ryegrass, giant foxtail, or johnsongrass. Results also suggest that *C. nebraskensis* cannot colonize johnsongrass rhizomes. The only study that tested seed transmission of C. *nebraskensis* in corn utilized a susceptible hybrid (Biddle et al. 1990). Campbell (2017) reported that alternative hosts of *C. nebraskensis* were more similar in symptom development to a moderately tolerant corn hybrid than a susceptible hybrid. Given that similarity, it can be expected that transmission of bacteria into plant seed would be similar, and the rate of *C. nebraskensis* seed transmission in resistant corn hybrids has not been reported.

4.5 Conclusion

We found that *C. nebraskensis* is able to overwinter on corn, giant foxtail, and large crabgrass debris on the soil surface and buried 15 cm in the soil in Indiana. This is the first report of *C. nebraskensis* overwintering on alternative host debris. Pathogenic bacteria were not detected in any plant debris starting 8 months after debris were buried or placed on the soil surface. This suggests that populations of pathogenic *C. nebraskensis* declined to levels where successful infection is not possible sometime between 4 and 8 months after plot establishment. Schuster (1975) reported that pathogenic *C. nebraskensis* can survive on all corn parts on the soil surface, and corn stems buried 10 and 20 cm deep, for up to 10 months in Nebraska. Our results suggest that the bacteria cannot survive that long in infested corn or alternative host debris in an Indiana environment and soil type. Several studies have reported that epiphytic populations of *C. nebraskensis* are found on corn plants early in the growing season without any symptoms of disease (Mallowa et al. 2016; Smidt and Vidaver 1986b). This suggests that populations in Indiana could live epiphytically on

actively growing corn or alternative hosts after overwintering in infected host debris. The cycle of *C. nebraskensis* transferring from debris to living tissue can help the pathogen survive multiple years in a field as long as a suitable host is present. Since we could not detect pathogenic *C. nebraskensis* in plant debris after the 4 month sampling timing, epiphytic populations on hosts during the growing season may be more important than previously thought for pathogen survival. Eggenberger et al. (2016) theorized that *C. nebraskensis* transferring from infested debris to corn in small areas in fields at low populations, over multiple years, can allow the pathogen to go unnoticed until a severe Goss's wilt outbreak is detected. Campbell (2017) found that 57 percent of Indiana fields surveyed with a history of Goss's wilt practice continuous corn crop rotation. He also found giant foxtail and large crabgrass in 94 and 63 percent of fields surveyed with a history of Goss's wilt, respectively. This indicates that many fields have hosts of *C. nebraskensis* present where bacteria could live epiphytically or infect hosts at imperceptible levels in a given year. Given our results about the ability to overwinter on host debris, this reinforces the importance of crop rotation and alternative host control in Goss's wilt management.

Results from these studies indicate that *C. nebraskensis* can overwinter on both corn and alternative host debris for equivalent times in Indiana. We were not able to detect populations of *C. nebraskensis* in the seed of annual ryegrass, giant foxtail, or johnsongrass that were inoculated up to three times throughout their life cycle. We also did not find any *C. nebraskensis* in johnsongrass rhizomes. These results are beneficial for the management of Goss's wilt due to potential implications of long-term survival due to inherent dormancy in seed of those species. It is also important since it indicates alternative hosts do not contribute long-distance transport of *C. nebraskensis* due to potential weed seed

transportation through equipment or wildlife, or annual ryegrass being sold as a cover crop across the US. Annual ryegrass is of particular concern for issues related to long-distance transport of seed since this species is used on approximately 37,000 hectares as a cover crop in the US (CTIC 2017). Overall, results indicate that cultural practices of crop rotation and alternative host control, in addition to selecting resistant corn hybrids, remain important disease management practices for Goss's wilt.

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Table 4.1. Average daily air temperature and cumulative precipitation for each month during the experimental period at the Agronomy Center for Research and Education near West Lafayette, IN in from December 2013 until December 2015.

Month	2013		2014		2015	
	Temp[a]	Precip	Temp	Precip	Temp	Precip
	C	cm	C	cm	C	cm
January			-8	4.80	-4	5.29
February			-7	12.09	-7	2.96
March			0	7.82	2	6.02
April			11	5.64	11	5.64
May			17	8.15	18	8.81
June			23	7.75	21	19.86
July			20	7.77	22	8.61
August			22	16.99	22	2.77
September			17	12.85	20	7.80
October			12	9.96	13	2.41
November			2	3.46	8	5.32
December	-2	13.54	1	5.96	5	20.90

[a]Abbreviations: Temp = temperature; Precip = precipitation.

Table 4.2. Percentage of plants sampled at each sampling timing with positive test results using Agdia Immunostrips® in the burial study.

Residue type	Burial depth	Sampling timing (months)						
		0	4	8	12	16	20	24
	cm	----------	----------	----------	%----------	----------	----------	----------
Corn leaf	0	100	100	100	100	NA	NA	NA
	15	100	100	NA[a]	NA	NA	NA	NA
Corn stem	0	100	100	100	100	0	0	0
	15	100	100	25	25	0	0	NA
Giant foxtail	0	100	100	0	25	0	0	0
	15	100	100	NA	NA	NA	NA	NA
Large crabgrass	0	100	100	50	25	0	0	0
	15	100	100	0	NA	NA	NA	NA

[a]Abbreviations: NA = Not applicable due to no residue being left to test in these treatments.

Table 4.3. Percent of inoculated corn plants with typical symptoms of Goss's wilt after inoculation with recovered bacteria for each plant sample and sample timing in burial study.

Residue type	Burial depth	Sampling timing (months)						
		0	4	8	12	16	20	24
	cm	----------%[a]----------						
Corn leaf	0	100	100	0	0	NA	NA	NA
	15	100	100	NA[b]	NA	NA	NA	NA
Corn stem	0	100	100	0	0	0	0	0
	15	100	100	0	0	0	0	NA
Giant foxtail	0	100	100	0	0	0	0	0
	15	100	100	NA	NA	NA	NA	NA
Large crabgrass	0	100	100	0	0	0	0	0
	15	100	100	0	NA	NA	NA	NA

[a]This represents the percentage of inoculated corn plants (out of ten) that exhibited typical symptoms of Goss's wilt for each residue type by sampling timing.

[b]Abbreviations: NA = Not applicable due to no residue being left to test in these treatments.

Table 4.4. Visual disease severity ratings for giant foxtail at 14 days after inoculation in seed transmission study. Inoculation timings were pooled across treatments to compare disease severity between inoculation timings.

Inoculation timing	Experiment one[a]	Experiment two
	----------%----------	
Three collar	17 A	6 B
Six collar	9 B	10 A
Seedhead emergence	1 C	4 C

[a]Mean values separated using Tukey's LSD. Values followed by different letters within column are significantly different ($P \leq 0.05$).

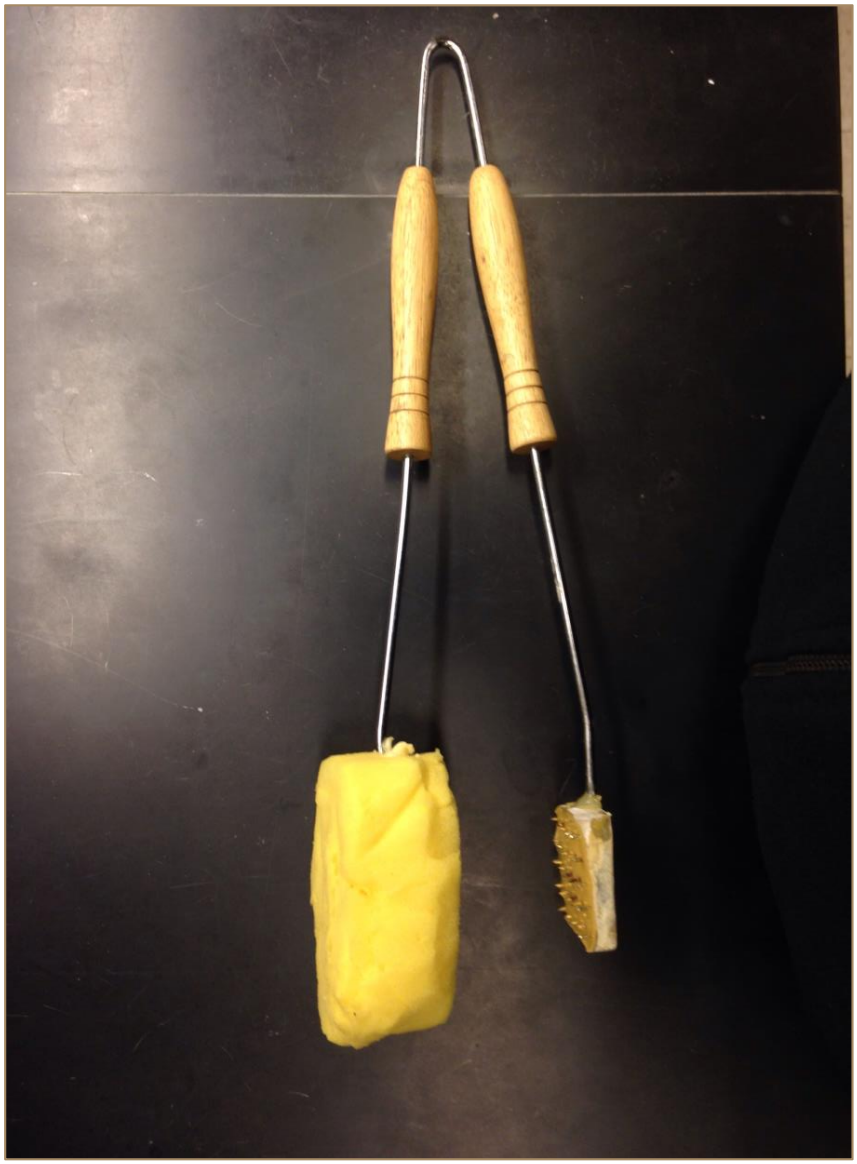

Figure 4.1. Modified inoculation device used in this study. The 5x5 grid of thumbtacks injures corn leaves, while sponge simultaneously delivers inoculum.

Figure 4.2. Map of the field layout for burial study at the agronomy Center for Research and Education near West Lafayette, IN. (A) White squares representing the location of each replication where samples were established. The southwest (lower left) replication is located within a different soil type. (B) United States Geological Survey soil map that shows two soil types in the field. Du = Drummer silty clay loam is represented in most of the field. RuB = Raub-Brenton silt loam is represented inside the orange polygon.

Figure 4.3. Modified inoculation device used in this study. The 5x1 grid of thumbtacks injures small leaves, while sponge simultaneously delivers inoculum.

CHAPTER 5. EFFECT OF POSTEMERGENCE HERBICIDES ON SEVERITY OF GOSS'S WILT IN CORN

5.1 Abstract

Goss's bacterial wilt and leaf blight of corn is caused by the bacterium *Clavibacter nebraskensis*. The disease re-emerged in 2006 and has subsequently spread across the Midwestern United States. The cause of re-emergence and spread of the disease is unknown, but has been attributed to an increase in hectares planted to continuous corn, an increase in no-tillage practices, and wide-spread use of corn hybrids that are susceptible to *C. nebraskensis*. Some have suggested that herbicide applications may play a role in the increased cases of Goss's wilt. In 2014 and 2016, field experiments were established at the Agronomy Center for Research and Education near West Lafayette, Indiana to determine if choice of single mode of action POST herbicide affected Goss's wilt severity. Six-row wide plots were established with the middle two rows containing a *C. nebraskensis*-susceptible corn hybrid and the outer four rows containing a *C. nebraskensis*-resistant hybrid. Only the middle two rows were inoculated with *C. nebraskensis* and the outer four rows served as borders to prevent the bacteria from spreading between plots. The middle two rows were inoculated at the V4 growth stage with a bacterial suspension containing 1 x 10^6 colony-forming units (CFU) of *C. nebraskensis* per mL. At the V6 growth stage, disease severity was measured on 10 plants per plot, then POST herbicide treatments were applied. Disease severity was measured on the same 10 plants per plot every two weeks until crop maturity to calculate the area under disease progress curve (AUDPC). After POST application, all plots were kept weed free until crop maturity, and grain yield was collected. Means were separated using Tukey's least significant differences with a $P \leq 0.05$.

In 2014, dicamba plus diflufenzopyr, glyphosate at 2520 g ha^{-1}, and nicosulfuron caused increased disease severity compared to the no herbicide control. There were no differences in percentage of plants with systemic wilt or yield in 2014. In 2016, there were no differences in disease severity, percentage of plants with systemic wilt, or yield. Results suggest that dicamba plus diflufenzopyr and nicosulfuron (both formulated with the safener isoxadifen-ethyl) applied at field use rates, and glyphosate applied at 2X the field use rate can increase Goss's wilt severity in corn without a subsequent yield loss. No herbicide treatment decreased the severity of Goss's wilt compared to the no herbicide control, indicating that herbicides cannot help control this disease.

5.2 Introduction

Goss's bacterial wilt and leaf blight (Goss's wilt) was first discovered in a hybrid corn (*Zea mays* L.) field in Nebraska in 1969 (Schuster et al. 1972). It is caused by the Gram positive bacterium *Clavibacter nebraskensis* comb. nov. (Li et al. 2018). The disease was widespread in the western corn belt throughout the 1970's, but only sporadic cases were reported from the 1980's until the disease re-emerged in the western corn belt in 2006 (Jackson et al. 2007). Since re-emergence, the disease has been found in 16 states in the United States (US): Colorado, Illinois, Indiana, Iowa, Kansas, Louisiana, Minnesota, Missouri, Nebraska, New Mexico, North Dakota, Oklahoma, South Dakota, Texas, Wisconsin, and Wyoming (Friskop et al. 2014; Hosack et al. 2016; Jackson et al. 2007; Korus et al. 2011; Malvick et al. 2010; Ruhl et al. 2009; Schuster 1975; Singh et al. 2015; Wysong et al. 1973; Yasuhara-Bell et al. 2016). Goss's wilt was recently cited as the third-leading cause of yield loss in corn due to disease in the Midwestern US and Ontario, Canada, where it caused an estimated loss of 13 million metric tons of corn yield in 2012

through 2015 (Mueller et al. 2016). Yield losses up to 44 percent have been documented in field trials, indicating the potential for severe yield loss in individual fields (Carson and Wicks III 1991).

The exact cause of re-emergence and subsequent spread to new areas is unknown, but has been attributed to an increase in corn-on-corn cropping practices, an increase in conservation tillage, and widespread planting of susceptible corn hybrids (Jackson et al. 2007). There is potential for long-distance transport of this pathogen through infected seed, though it is found in seed at very low rates (Biddle et al. 1990). Langemeier et al. (2017) conducted a targeted survey of agronomic practices across the western Corn Belt to determine agronomic factors associated with Goss's wilt. They found that fields planted to a *C. nebraskensis*-susceptible hybrid with a population over 67,500 seed ha^{-1} had confirmed incidence of Goss's wilt in 88 percent of surveyed fields. They also found a positive correlation between fields with Goss's wilt and fields that had glyphosate applied to them. They suggested this relationship might be because 70 percent of fields surveyed received a glyphosate application, regardless of Goss's wilt incidence in those fields. This reflects a relatively high use rate of glyphosate in glyphosate-resistant (GR) corn. Genetically engineered herbicide-tolerant corn was planted on 72 percent of US corn acres in 2011 when Langemeier et al. (2017) conducted this survey (USDA-ERS 2018). This marks a significant increase in planting of herbicide tolerant corn in the US compared to when Goss's wilt re-emerged in 2006 (36 percent of corn acres). Without experimental evidence to determine any cause of the correlation between glyphosate and Goss's wilt incidence, the authors could not determine if glyphosate actually influenced disease infection and severity in surveyed fields.

Several studies have been conducted on the effects of herbicide applications on incidence or severity on plant diseases. Altman and Campbell (1977) reviewed the available literature of approximately 60 herbicide active ingredients and their effects on plant diseases. They found several studies where herbicide applications increased disease, as well as several studies where herbicide applications decreased disease. Of note, most of these studies were conducted in the lab or greenhouse, with no experimental evidence from field trials. Many studies also did not identify the causal agent of plant diseases, or the mechanism of decreased or increased disease. Herbicide use patterns have changed since their review with the release of several new active ingredients and modes of action. In particular, the increased use of glyphosate since the release of GR crops has shifted use patterns of herbicides since pre-adoption of GR crops, with the use of glyphosate over the top in GR crops representing the most dramatic shift in use pattern (Young 2006). Johal and Huber (2009) reviewed studies that associated increased disease with glyphosate weed control programs, though they did not report any cases of increased corn disease associated with glyphosate use. They speculated that glyphosate toxicity in glyphosate-resistant crops would increase their vulnerability to pathogens after glyphosate application. In contrast, Duke et al. (2012) suggested that disease susceptibility in herbicide-tolerant crops is driven by inherent susceptibility to the pathogen in the host crops, and not the insertion of the herbicide tolerant genes.

There has been speculation that use of glyphosate, other herbicides, or their associated adjuvants has led to an increase in plant disease, specifically those caused by *Clavibacter* species (Huber 2011). Schlund (2015) tested the effect of the most commonly used adjuvants on Goss's wilt and found no difference in disease severity or corn yield.

Williams et al. (2015) found that application of glyphosate to transgenic sweet corn both prior to and following *C. nebraskensis* infection resulted in the same levels of Goss's wilt incidence as inoculated plants that did not receive a glyphosate application. There was also no difference in Goss's wilt incidence between GR and glyphosate-susceptible corn lines, suggesting that Goss's wilt incidence is driven by susceptible genes in different corn lines, and not attributed to the insertion of a GR gene nor the application of glyphosate. To date, glyphosate has been the only herbicide tested for effects on Goss's wilt incidence or severity in corn. The objective of our study was to apply different single active ingredient herbicides representing the most commonly used modes of action in corn production on *C. nebraskensis*-infected corn to measure any responses in disease severity throughout the growing season or corn yield.

5.3 Materials and Methods

5.3.1 Inoculation Preparation

A confirmed strain of *C. nebraskensis* that was isolated from Pulaski county Indiana in 2008 and stored in a 20 percent glycerol solution at minus 80 C was used for inoculation in this study (Ikley et al. 2015). The isolate was grown on Nutrient Broth Yeast (NBY) media for 3 d at 24 C. Bacterial colonies were suspended in sterile double-distilled water (ddH_2O). Inoculum concentration was adjusted to a concentration of 1 x 10^6 colony-forming units (CFU) per ml with a Spectrophotometer (Beckman Coulter DU 530, Beckman Coulter, Inc., Brea, CA) set at 600 nanometers (nm). A concentration of 1 x 10^6 CFU per ml was selected in order to ensure successful infection prior to herbicide application and because it provides sufficient disease severity for testing (Calub et al. 1974).

5.3.2 Field Sites

Experiments were initiated in June of 2014 and May of 2016 at the Agronomy Center for Research and Education (ACRE) near West Lafayette, IN. Field sites consisted of a Drummer Soil (silty clay loam) that were conventionally tilled, with chisel plowing in the previous fall and field cultivation as needed for planting in the spring. Corn was planted in 76-cm rows on June 26, 2014, and May 21, 2016 at a rate of 80,000 seed ha^{-1}. Weather conditions throughout each growing season are located in Table 5.1. A preemergence herbicide application of the premix Lexar® EZ (Syngenta Crop Protection, Basel, Switzerland) was applied at a rate of 5.26 L ha^{-1}, resulting in 0.14 kg ha^{-1} mesotrione, 1.1 kg ha^{-1} *s*-metolachlor, and 1.1 kg ha^{-1} atrazine.

5.3.3 Experimental Design and Herbicide Applications

Plots contained six corn rows, measured 4.5 m in width, and 9 m in length, and were arranged in a randomized complete block design with four replications. Within each plot, the center two rows were a *C. nebraskensis*-susceptible corn hybrid (DKC55-09RIB Brand Blend, Dekalb® Corn, Monsanto Company, St. Louis, MO), and the outer four rows were rated as tolerant to *C. nebraskensis* (5939VT3PRIB, Great Lakes Hybrid, LG Seeds, Westfield, IN). Every plant in the center two rows were inoculated once plants had 4 visible collars (V4 growth stage) using a modified pin-prick device (Figure 5.1). This layout ensured there were four rows of a tolerant hybrid in between the two inoculated susceptible rows for each plot. Inoculation occurred on July 21, 2014, and June 17, 2016. The sponge end of the device was dipped into the inoculum, excess inoculum was squeezed out of the sponge, and then the top two fully expanded corn leaves of two consecutive plants were inoculated by squeezing the leaves in between the sponge and the thumb-tacks, which

caused wounds while the sponge simultaneously delivered approximately 6 ml of inoculum to each injured leaf.

Seven days after inoculation when plants had 6 visible collars (V6 growth stage), 10 individual plants per plot were rated for percent symptomatic leaf tissue of the inoculated leaves. These same plants were marked and rated throughout the season in order to develop an Area Under Disease Progress Curve (AUDPC). After this initial rating, 9 different herbicide treatments and a no-herbicide control were applied to the plots (Table 5.2). Adjuvants were tank-mixed with herbicides based on instructions found on the herbicide labels (Table 5.2). Sources of adjuvants were as follows: Ammonium sulfate (AMS; N-Pak, Winfield Solutions, LLC, St. Paul, MN) was added at 5% v/v, which resulted in 2% w/v active ingredient AMS per tank mixture. Crop oil concentrate (COC; Prime Oil, Winfield Solutions, LLC, St. Paul, MN) was added at 1% v/v. Methylated seed oil (MSO Ultra, Precision Laboratories, LLC, Waukegan, IL) was added at 1% v/v. Nonionic surfactant (Activator-90, Loveland Products Inc., Loveland, CO) was added at 0.25% v/v. Herbicides were applied to the center 1.5 m of each plot with a handheld CO_2-pressurized backpack sprayer calibrated to deliver 140 L ha^{-1} at a speed of 4.8 km h^{-1} with XR8002 nozzles in 38-cm spacing. The no herbicide control was hand-weeded at this time. All plots were hand-weeded every two weeks afterwards to ensure all plots remained weed-free until harvest.

Treatments in Table 5.2 consisted of herbicides with different modes of action. These herbicides were selected because they are widely used postemergence herbicides in Indiana corn production within their respective modes of action. In addition to seven treatments of unique modes of action at typical field use rates, two additional treatments

were included. These treatments were glyphosate at 2520 g ha^{-1} (2X the labeled use rate), and the treatment containing dicamba plus diflufenzopyr. Dicamba plus diflufenzopyr was included because that herbicide (Status®: BASF, Research Triangle Park, NC) is widely used in Indiana, and diflufenzopyr was not available as solo product at the time of this study's initiation. The 2X rate of glyphosate was included due to glyphosate's implication with Goss's wilt incidence across the Midwest (Langemeier et al. 2017).

5.3.4 Data Collection and Analysis

The 10 individually marked corn plants were rated every 2 weeks throughout the growing season for Goss's wilt disease severity. Plants were rated by visually estimating the average percent of symptomatic tissue (0 to 100 scale, with 0 representing no infection, and 100 representing complete leaf death) per leaf and multiplying by the percentage of total infected leaves per plant. Ratings were taken until leaf senescence, which took place between 8 and 10 weeks after herbicide treatment in each experiment. These disease ratings were then used to calculate the AUDPC for each plant throughout the growing season. AUDPC calculation was: $\sum_{i=1}^{N_i-1}\left(\frac{y_i+y_{i+1}}{2}\right)(t_{i+1}-t_i)$, where t_i represents sample timing in days after inoculation, and y_i represents disease severity at that sample timing. At 6 weeks after herbicide application, the number of plants per plot that exhibited the systemic wilt phase of Goss's wilt was determined. In both years, plants that had visual symptoms of systemic infection at 6 weeks after herbicide treatment did not produce a harvestable ear due to entire plant death (*Data not shown*). The center two rows of plots were harvested on January 15, 2015 and October 14, 2016 with a Kincaid 8-XP combine (Kincaid Equipment Manufacturing, Haven KS). Plot weights and moisture were measured during harvest, were standardized to 15.5 percent moisture, and converted from lb $plot^{-1}$ to kg ha^{-1}.

Percent disease severity was calculated into AUDPC and analyzed for difference between treatments using a repeated measurements analysis of variance with the PROC MIXED procedure in SAS 9.4 (SAS Institute, Inc., Cary, NC) with sample timing as the repeated measure. Where differences occurred means were separated with Tukey's honestly significant difference (HSD) at P=0.05. The percent of plants exhibiting symptoms of the systemic wilt phase of the disease were analyzed using ANOVA with SAS 9.4 using the PROC GLIMMIX procedure. Both disease severity and percent of plants exhibiting symptoms of systemic infection had treatment by year interactions (P=0.0032 and P=0.0481, respectively), so they were analyzed separately by year.

The *C. nebraskensis*-tolerant hybrid was not glufosinate-tolerant, and did not survive the glufosinate herbicide application in either experiment. This afforded the middle two rows of the glufosinate treatment extra sunlight and less competition from neighboring plants compared to other herbicide treatments, and thus they were omitted from yield data analysis due to increased yield from an "edge effect". Yield was subjected to ANOVA using the PROC GLIMMIX procedure in SAS 9.4. There was no treatment by year interaction, so data we analyzed together (P=0.9514; Table 5.5). Data are also presented separately by year since all disease measurements were analyzed separately by year (Tables 5.3 and 5.4). Data for all measurements were checked for homogeneity of variance and normality using PROC UNIVARIATE in SAS 9.4. All data were normal and were not transformed for analysis.

5.4 Results and Discussion

5.4.1 Disease Severity and Yield

In 2014, there were differences in disease severity due to herbicide treatment (Table 5.3). Dicamba plus diflufenzopyr, glyphosate (2X), and nicosulfuron caused the most disease severity. All three treatments had increased disease severity compared to the no herbicide control. They were also different than atrazine, flumiclorac, glyphosate (1X), and topramezone. No other treatments were different from the no herbicide control. A contrast statement was also performed between the dicamba and dicamba plus diflufenzopyr treatments and they were different, with dicamba plus diflufenzopyr causing more disease then dicamba alone (P=0.0239). This suggests that the addition of the auxin transport inhibitor can increase disease severity even though the rate of dicamba is 15% of the dicamba alone treatment (84 vs 560 g ha^{-1}). Another explanation is that formulated dicamba plus diflufenzopyr (Status®) and nicosulfuron (Accent® Q) contain the herbicide safener isoxadifen-ethyl (Anonymous 2015, 2016). This chemical makes some herbicides less injurious to corn by increasing cytochrome P450 production in corn (Paporish and Rubin 2017; Rosinger 2014). Corn utilizes the same cytochrome P450s to metabolize dicamba plus diflufenzopyr, nicosulfuron, and topramezone (Pataky et al. 2011). Topramezone is not formulated with isoxadifen-ethyl, did not increase disease severity compared to the no herbicide control, and it caused less disease severity than dicamba plus diflufenzopyr and nicosulfuron in this experiment (Anonymous 2017; Table 5.3). The exact mechanism of isoxadifen-ethyl increasing cytochrome P450s in corn is unknown. In addition to increasing cytochrome P450s, up to 446 genes are differentially regulated by isoxadifen-ethyl in Arabidopsis for the purposes of xenobiotic detoxification (Behringer et al. 2011).

Perhaps as these genes are differentially regulated, unknown plant pathogen defenses are inhibited or downregulated enough for *C. nebraskensis* to cause more disease in corn.

There were no differences between treatments in the percent of plants per plot exhibiting systemic wilt phase in 2014. Using contrast statements did not reveal any differences between dicamba compared to dicamba plus diflufenzopyr (P=0.4018) nor glyphosate (1X) compared to glyphosate (2X) (P=0.3017). There were also no differences in yield between treatments in 2014. Contrast statements between dicamba compared to dicamba plus diflufenzopyr (P=0.2797) and glyphosate (1X) compared to glyphosate (2X) (P=0.5005) also revealed no differences. These data suggest that even though differences were observed in disease severity, the increased disease severity of dicamba plus diflufenzopyr, glyphosate (2X), and nicosulfuron did not affect the number of plants that failed to produce a harvestable ear due to the systemic wilt phase of the Goss's wilt, or corn yield in 2014.

There was greater disease severity, more plants with systemic wilt, and lower corn yield in 2016 compared to 2014 (Tables 5.3 and 5.4). This is likely explained by hotter temperatures throughout the growing season following inoculation in 2016 compared to 2014 (Figure 5.2). Vidaver and Mandel (1974) reported that *C. nebraskensis* populations grow most rapidly in temperatures between 24 C and 28 C. Smidt and Vidaver (1986) determined that the optimal temperature for growth of *C. nebraskensis* is 27 C. The warmer temperatures in 2016 resulted in more time during the growing season with air temperatures closer to that optimal temperature. Precipitation was similar between the two experiments (29.92 cm in 2014 vs. 29.69 cm in 2016) but the warmer average low temperatures throughout 2016 also suggests higher humidity levels throughout that experiment. Mallowa

et al. (2016) found that Goss's wilt leaf blight symptoms are expressed at higher levels in high humidity environments.

In 2016, there were no differences between treatments with regards to disease severity, percent of plants with systemic wilt, or corn yield (Table 5.4). Contrast statements also reveal no differences in disease severity, number of plants with systemic wilt or yield for dicamba compared to dicamba plus diflufenzopyr (P=0.0607, P=0.0908, and P=0.7967, respectively). Contrasts for glyphosate (1X) compared to glyphosate (2X) revealed a difference in the percentage of plants with systemic wilt (P=0.0430), but no differences in disease severity or yield (P=0.5736 and P=0.4687, respectively). The higher levels of disease severity in 2016 could be the reason results were different than 2014. A more favorable environment led to higher disease pressure, which could have been a more important factor than herbicide treatments in determining disease severity or yield. Overall, yields were very low in 2016. We did not include a non-inoculated control to compare yield against, but the same variety (DKC 55-09 RIB) planted in the same field on the same day averaged 11,300 kg ha^{-1} when kept weed free with no disease in a separate study (Campbell 2017). Average yields were approximately 9,000 kg ha^{-1} lower than that in this study across all treatments, representing a 79 percent yield loss due to Goss's wilt. This is a greater yield loss due to *C. nebraskensis* than the 44 percent yield loss reported by Carson and Wicks III (1991).

We were able to combine yields across 2014 and 2016 for analysis due to no treatment by year effect (P=0.9514). We saw no differences in yield between treatments across years. Contrast statements also reveal no difference between dicamba and dicamba plus diflufenzopyr (P=0.3313) or glyphosate (1X) compared to glyphosate (2X)

(P=0.2937). This shows that the differences in disease severity between treatments in the two years did not affect corn yield.

5.5 Conclusions

Results from this study show that herbicide treatment following *C. nebraskensis* infection in corn did not influence yield. This allows for flexibility in choosing a herbicide program to target a field's weed spectrum without having to worry about a yield loss due to increased disease pressure. We did observe that in a lower disease pressure year, dicamba plus diflufenzopyr and nicosulfuron at field use rates can increase disease severity without a yield penalty. The influence of the safener isoxadifen-ethyl should be investigated further to see if it downregulates pathogen protection genes while increasing the number of cytochrome P450s in order help corn metabolize these herbicides. Glyphosate (2X) also increased disease severity without affecting yield. This highlights the importance of proper sprayer calibration and operation to avoid overlaps that would result in a 2X rate of glyphosate. These results are important because increased disease severity does increase inoculum available for infection in future years. Eggenenberger et al. (2016) suggested that outbreaks of Goss's wilt in recent years have come from unnoticeable buildup of *C. nebraskensis* inoculum in fields. They found that Goss's wilt incidence is most likely to occur in plants with nearby point sources of inoculum like crop residue. If areas in a field received a 2X rate of glyphosate that led to increased disease severity, these could be areas of a field where disease outbreak would be more likely compared to the rest of the field that received the labeled rate due to increased inoculum.

Managing inoculum levels is important for disease management of *C. nebraskensis* since cultural practices remain the only effective strategies for management. Our results

also showed that no treatments decreased disease severity from the no herbicide control, suggesting that commercially available herbicidal modes of action for use in corn cannot decrease disease severity. Previous research has suggested that atrazine and glufosinate have antimicrobial activity against some plant pathogens, but this did not prove true for *C. nebraskensis* in this study (Pline et al. 2001; Sanyal and Shrestha 2008). Schlund (2015) found that adjuvants, fungicides, and copper hydroxide also did not decrease disease severity when applied to *C. nebraskesnsis*-infected corn plants. Higher than labeled rates of some adjuvants tested proved phytotoxic to *C. nebraskensis* in laboratory testing, but these results did not translate *C. nebraskensis* populations inside corn plants. With no effective chemical control measures, this means cultural practices like crop rotation, tillage, hybrid selection, and control of alternative hosts remain the best method to reduce inoculum levels and avoid disease incidence of Goss's wilt.

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Table 5.1. Average minimum temperature, average maximum temperature, average daily air temperature, and cumulative precipitation for each month during the experimental period at the Agronomy Center for Research and Education near West Lafayette, IN in 2014 and 2016.[a]

Month	2014				2016			
	Avg min temp	Avg max temp	Avg daily temp	Precipitation	Avg min temp	Avg max temp	Avg daily temp	Precipitation
	C	C	C	cm	C	C	C	cm
May	11	23	17	8.15	8	23	16	4.34
June	16	29	23	7.75	16	30	23	11.94
July	14	27	20	7.77	17	29	23	12.80
August	16	29	22	16.99	18	30	24	15.47
September	10	25	17	12.85	14	28	21	8.10
October	6	18	12	9.96	7	22	15	3.28
November	-3	6	2	3.46	3	15	8	13.59
December	-2	5	1	5.96	-4	2	-1	5.87

[a]Abbreviations: Min = minimum; Max = maximum; Avg = average; temp = temperature.

Table 5.2. Sources of commercial herbicides used in field experiment.

Common name	Trade name	Manufacturer	Mode of action	Rate	Adjuvants used[a]
				g ha^{-1}	
Atrazine	Aatrex® 4L	Syngenta Crop Protection, LLC	Photosystem II inhibitor	1680	COC
Dicamba	Clarity®	BASF Corp.	TIR1 auxin receptor	560	AMS
Dicamba+ diflufenzopyr	Status®	BASF Corp.	TIR1 auxin receptor + auxin transport inhibitor	84 + 34	AMS + NIS
Flumiclorac	Resource®	Valent USA Corp.	PPO inhibitor	37.7	COC
Glufosinate	Liberty® 280 SL	Bayer CropScience, LP	Glutamine synthase inhibitor	593	AMS
Glyphosate	Roundup® Powermax	Monsanto Co.	EPSPS inhibitor	1260	AMS
Glyphosate	Roundup® Powermax	Monsanto Co.	EPSPS inhibitor	2520	AMS
Nicosulfuron	Accent® Q	DuPont	ALS inhibitor	35	AMS + COC
Topramezone	Armezon™	BASF Corp.	HPPD inhibitor	18.4	AMS + MSO

[a] Abbreviations: AMS = ammonium sulfate; COC = crop oil concentrate; MSO = methylated seed oil; NIS = non-ionic surfactant.

Table 5.3. Disease severity reported as area under disease progress curve (AUDPC), percentage of plants showing symptoms of systemic infection, and yield response to herbicide treatments applied to *C. nebraskensis*-infected corn plants at the Agronomy Center for Research and Education in 2014.

Common name	Rate	AUDPC[a]	Systemic plants	Yield
	g ha^{-1}		%	Kg ha^{-1}
Atrazine	1680	1042 B	7	5178
Dicamba	560	1239 AB	12	5097
Dicamba+ diflufenzopyr	84 + 34	1472 A	16	4523
Flumiclorac	37.7	1056 B	7	5278
Glufosinate	593	1160 AB	9	NA
Glyphosate	1260	916 B	5	5349
Glyphosate	2520	1430 A	10	4993
Nicosulfuron	35	1267 A	7	5412
Topramezone	18.4	958 B	5	5775
No herbicide	0	981 B	8	5630

[a]Mean values separated using Tukey's HSD. Values followed by different letters within column are significantly different ($P \leq 0.05$).

Table 5.4. Disease severity reported as area under disease progress curve (AUDPC), percentage of plants showing symptoms of systemic infection, and yield response to herbicide treatments applied to *C. nebraskensis*-infected corn plants at the Agronomy Center for Research and Education in 2016.

Common name	Rate	AUDPC[a]	Systemic plants	Yield
	g ha^{-1}		%	Kg ha^{-1}
Atrazine	1680	2689	47	2334
Dicamba	560	2273	24	2088
Dicamba+ diflufenzopyr	84 + 34	2563	37	1935
Flumiclorac	37.7	2239	22	2821
Glufosinate	593	2421	23	NA
Glyphosate	1260	2301	29	2447
Glyphosate	2520	2387	45	2016
Nicosulfuron	35	2248	27	2129
Topramezone	18.4	2281	37	2295
No herbicide	0	2474	30	2560

[a]Tukey's HSD (0.05) = not significant for all response variables tested.

Table 5.5. Yield response to herbicide treatments applied to *C. nebraskensis*-infected corn plants at the Agronomy Center for Research and Education in 2014 and 2016.

Common name	Rate	Yield[a]
	g ha^{-1}	Kg ha^{-1}
Atrazine	1680	3756
Dicamba	560	3592
Dicamba+ diflufenzopyr	84 + 34	3229
Flumiclorac	37.7	4050
Glufosinate	593	NA
Glyphosate	1260	3898
Glyphosate	2520	3505
Nicosulfuron	35	3770
Topramezone	18.4	4035
No herbicide	0	4095

[a]Tukey's HSD (0.05) = not significant for all response variables tested.

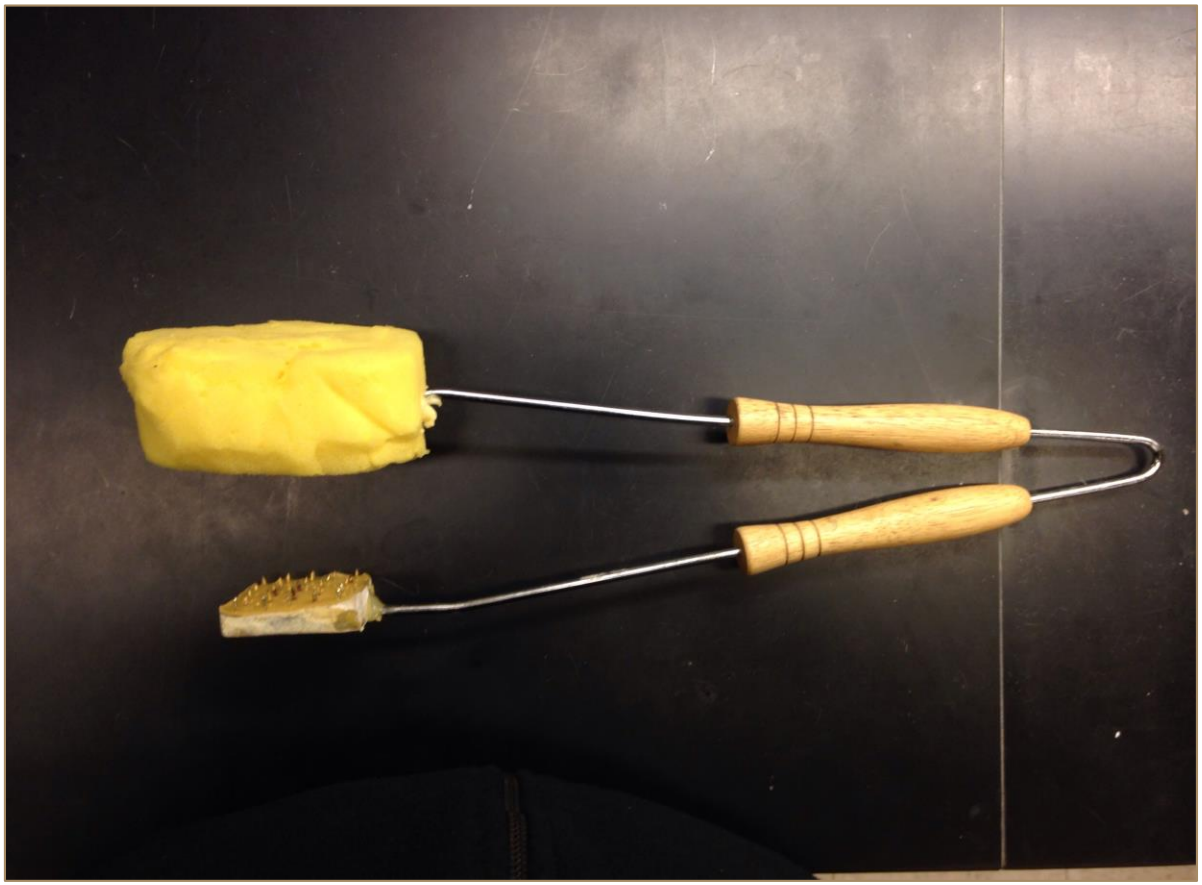

Figure 5.1. Modified inoculation device used in this study. The 5x5 grid of thumbtacks is used to injure corn leaves, while sponge simultaneously delivers inoculum.

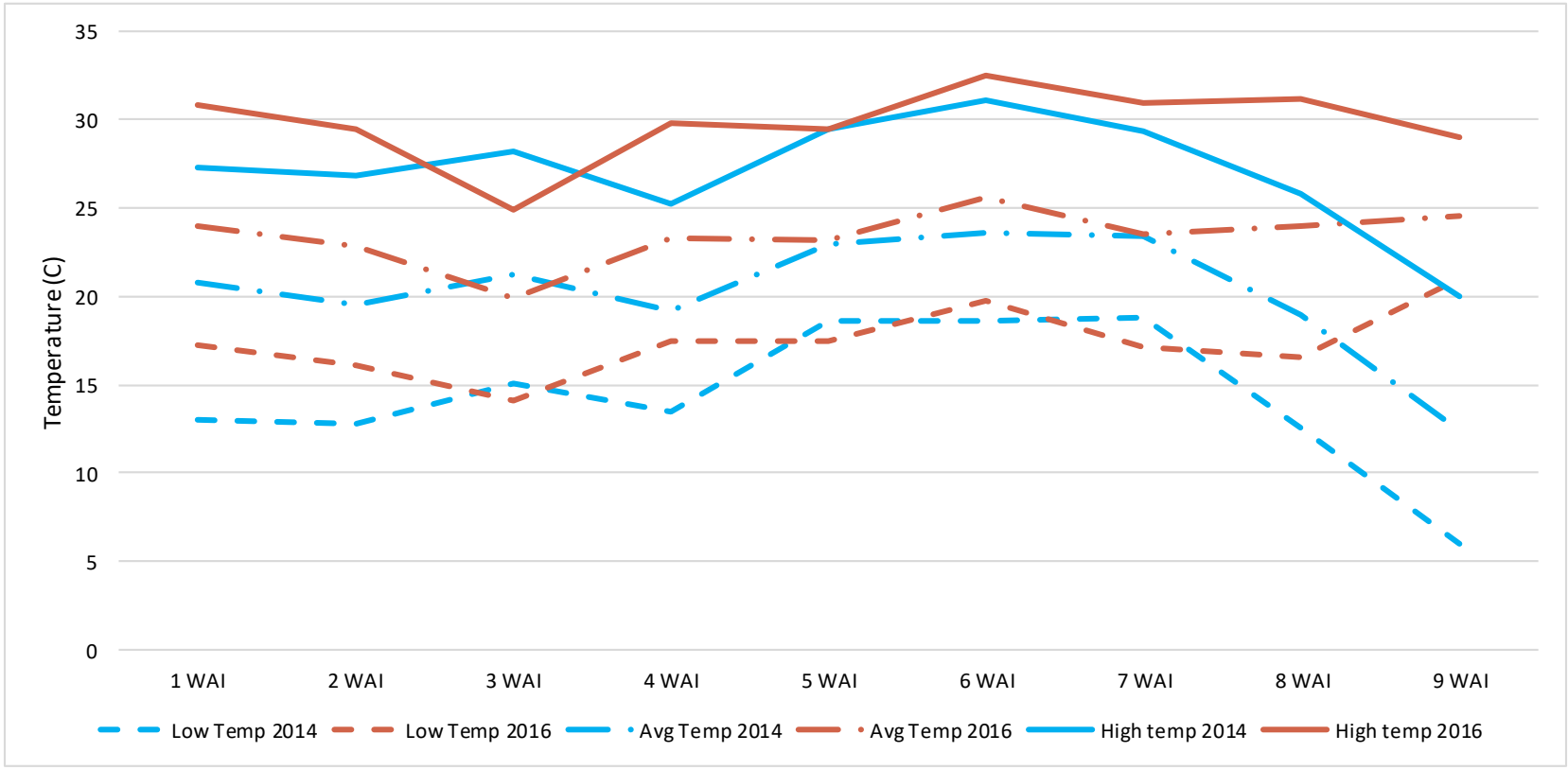

Figure 5.2. Average minimum temperature, average maximum temperature, and average daily air temperature for the 9 weeks after inoculation during the disease severity measurement period at the Agronomy Center for Research and Education near West Lafayette, IN in 2014 and 2016. Abreviations: WAI = weeks after inoculation; temp =temperature; avg = average.

CHAPTER 6. THE EFFECT OF INOCULATION TIMING AND ANNUAL RYEGRASS VARIETY ON TRANSMISSION OF *C. NEBRASKENSIS* TO CORN

6.1 Abstract

Annual ryegrass (*Lolium multiflorum* L.) is an alternative host for *Clavibacter nebraskensis*, the causal agent of Goss's wilt of corn. Annual ryegrass is planted as a cover crop on many hectares across the US Corn Belt. *C. nebraskensis* is documented to overwinter in corn debris, but the ability of the bacterium to overwinter an annual ryegrass is unknown. Corn hybrids vary in their susceptibility to *C. nebraskensis*, and are generally less susceptible as plants age. Field experiments were established to determine if there is a difference in annual ryegrass susceptibility to *C. nebraskensis* between different varieties, if susceptibility of annual ryegrass to *C. nebraskensis* changes as plants mature, and if the bacterium can transfer from annual ryegrass to corn. Annual ryegrass was seeded into standing corn approximately 4 weeks before harvest in 2014 and 2015. Treatments consisted of ryegrass being inoculated with a bacterial suspension containing 1 x 10^6 colony-forming units (CFU) of *C. nebraskensis* per ml once in the fall, and three separate times in the spring. Typical symptoms of *C. nebraskensis* infection in annual ryegrass were never observed throughout the course of this study. No symptoms of Goss's wilt were observed on corn throughout the study. Differences in corn yield were determined based on which annual ryegrass variety corn was planted into. Results indicate that *C. nebraskensis* cannot infect annual ryegrass between September and June in Indiana, and cannot transfer from annual ryegrass to corn. Results suggest varietal differences in annual ryegrass competition with corn that

caused yield loss in corn, though the mechanism of difference in competition was not elucidated in this study.

6.2 Introduction

Goss's bacterial wilt and leaf blight (Goss's wilt) was first discovered in a hybrid corn (*Zea mays* L.) field in Nebraska in 1969 (Vidaver and Mandel 1974). It is caused by the Gram positive bacterium *Clavibacter nebraskensis* comb. nov. (Li et al. 2018). The disease was widespread in the western corn belt throughout the 1970's, but only sporadic cases were reported from the 1980's until the disease re-emerged in the western corn belt in 2006 (Jackson et al. 2007). The disease has become increasingly important across the Midwestern US over the last decade (Mueller et al. 2016). The re-emergence has been attributed to an increase in continuous corn cropping practices, an increase in conservation tillage, and widespread planting of *C. nebraskensis*-susceptible corn hybrids (Jackson et al. 2007). *C. nebraskensis* can survive on corn leaf and stalk residue on the soil surface, and on buried corn stalks for up to 10 months (Schuster 1975). Infected corn debris is a main source of *C. nebraskensis* inoculum and contributes more to the spread of disease incidence within a field than bacterial spread between living corn plants (Eggenberger et al. 2016).

There are several alternative hosts to *C. nebraskensis*, though their role in Goss's wilt epidemiology is unknown. Commonly found weedy hosts of *C. nebraskensis* include bristly foxtail [*Setaria verticillata* (L.) Beauv.], giant foxtail (*Setaria faberi* Herm.), green foxtail [*Setaria viridis* (L.) Beauv.], yellow foxtail [*Setaria pumila* (Poir.) Roemer & J.A. Schultes], large crabgrass [*Digitaria sanguinalis* (L.)], shattercane [*Sorghum bicolor* (L.) Moench ssp. *Arundinaceum* (Desv.) de Wet & Harlan], johnsongrass [*Sorghum halepense* (L.) Pers.], and woolly cupgrass [*Eriochloa villosa (Thunb.) Kunth]* (Langemeier et al.

2014; Ikley et al. 2015, Webster 2016). Ikley et al. (2015) found the commonly used cover crop annual ryegrass (*Lolium multiflorum* Lam.) is also an alternative host. The use of cover crops has increased in Indiana over the last decade, and there are currently approximately 400,000 hectares planted annually in the state (Harmon 2017). The hectares of annual ryegrass planted as a cover crop have increased in recent years, and it is the only known cover crop host of *C. nebraskensis*. Only 5 other species of cover crop are planted on more hectares across the US. Annual ryegrass was planted on approximately 37,000 hectares in 2017 (CTIC 2017). The practice of widespread planting of an alternative hosts of *C. nebraskensis* is concerning due to the potential to increase inoculum in fields that might otherwise be fallow during the winter months. The presence of annual ryegrass could act as a "green bridge" and provide another overwintering source for *C. nebraskensis* other than plant debris. This could be particularly troublesome where corn is "planted green" into living cover crops. The cover crop cereal rye (*Secale cereale* L.) can increase the incidence of corn seedling diseases caused by *Pythium spp*. when the interval between cereal rye termination and corn planting is shortened. Archarya et al. (2017) found that corn seedling diseases were reduced when cereal rye was terminated two to three weeks prior to corn planting compared to termination timing closer to corn planting.

There are numerous varieties of annual ryegrass that have been developed and marketed for the cover crop market. Corn hybrids are known to have varying levels of susceptibility to *C. nebraskensis* (Calub et al. 1974a; Mbofung et al. 2016). Calubt et al. (1974b) demonstrated that corn becomes less susceptible to *C. nebraskensis* as plants mature. Annual ryegrass has not been tested for differences in susceptibility to *C. nebraskensis* between varieties or plant age. The objectives for this study were to (1)

determine if there is a difference in susceptibility to *C. nebraskensis* between three different annual ryegrass varieties grown in the field, (2) determine if there is a difference in susceptibility of annual ryegrass to *C. nebraskensis* as plant age increases, and (3) determine if *C. nebraskensis* is able to transfer to corn from inoculated annual ryegrass in the field.

6.3 Materials and Methods

6.3.1 Variety Selection and Inoculum Preparation

The annual ryegrass varieties were: Bruiser (AMPAC, Tangent, OR), Gulf (Oregon Grown, Albany OR), and Winter Hawk (Cisco Farm Seed, Indianapolis, IN). All three varieties are diploids that have been developed for cover crop markets. The *C. nebraskensis*-susceptible corn hybrid used was DKC 55-09 RIB (Dekalb Corn, Monsanto Company, St. Louis, MO) that is rated as an eight on a one-to-nine scale with nine being the most susceptible to the bacterium.

A confirmed strain of *C. nebraskensis* that was isolated from Pulaski county Indiana in 2008 and stored in a 20 percent glycerol solution at minus 80 C was obtained from the Purdue Plant and Pest Diagnostic Laboratory (Ruhl et al. 2009). The isolate was grown on Nutrient Broth Yeast (NBY) media for 3 d at 24 C. Bacterial colonies were suspended in sterile double-distilled water (ddH_2O). Inoculum concentration was adjusted to a concentration of 1 x 10^6 colony-forming units (CFU) of *C. nebraskensis* per ml with a Spectrophotometer (Beckman Coulter DU 530, Beckman Coulter, Inc., Brea, CA) set at 600 nanometers (nm).

6.3.2 Experimental Design and Inoculation

Field experiments were established in September 2014 and September 2015 at the Agronomy Center for Research and Education (ACRE) near West Lafayette IN. Experiments were a split-block design with four replications. Annual ryegrass variety was the main block, and treatments consisted of five different inoculation timings within each variety. The five inoculation timings were: (1) same day as corn harvest, (2) at spring green up, (3) at annual ryegrass bolting, (4) at annual ryegrass seedhead emergence, and (5) a non-inoculated control. Individual plots measured 3 m in width by 9 m in length

Annual ryegrass varieties were seeded with hand spreaders while walking through standing corn at approximately the milk (R3) reproductive growth stage. Hand spreaders were calibrated to deliver approximately 28 kg of annual ryegrass per hectare while walking at 3.2 kph. There were three methods used to inoculate annual ryegrass in these experiments. Wounds were created at corn harvest by corn debris being ejected from a chopper mounted on the rear of the combine prior to spraying inoculum on the plants. Plants were wounded at spring green up using a push mower with a 61-cm width deck pushed between corn rows and set to mow at a height of 10 cm prior to spraying inoculum on the plants. A modified pin-prick device was used at bolting and seedhead emergence to cause wounds in annual ryegrass plants (Figure 6.1). When pulled with a rope, this device caused numerous puncture and tear wounds on annual ryegrass plants. Plots were sprayed with inoculum directly after wounding.

The *C. nebraskensis*-susceptible corn hybrid was no-till planted into the living annual ryegrass on May 23, 2015 and May 21, 2016 in 76-cm rows, resulting in 4 rows per plot, at a population of 80,000 seed per hectare. Annual ryegrass was terminated two w after corn planting in each experiment with a tank-mix of glyphosate at 1260 g ae per ha

(Roundup Powermax, Monsanto Company, St. Louis, MO) plus the premixture Lexar® EZ (Syngenta Crop Protection, Basel, Switzerland) applied at a rate of 5.26 L ha^{-1}, resulting in 0.14 kg ha^{-1} mesotrione, 1.1 kg ha^{-1} *s*-metolachlor, and 1.1 kg ha^{-1} atrazine. Corn growth stage was one to two visible collars (V1 to V2) at herbicide application.

6.3.3 Data Collection and Analysis

Annual ryegrass varieties were evaluated for disease incidence at 14 d after inoculation, and at termination. Corn plants were evaluated for disease incidence every 2 w from emergence until maturity. The center two corn rows of each plot were harvested on October 6, 2015 and October 14, 2016 with a Kincaid 8-XP combine (Kincaid Equipment Manufacturing, Haven KS). Plot weights and moisture were measured during harvest, standardized to 15.5 percent moisture, and converted from lb $plot^{-1}$ to kg ha^{-1}.

No symptoms of infection from *C. nebraskensis* were ever observed on annual ryegrass or corn, so no disease incidence data were analyzed. Yield data were checked for normality and homogeneity of variance using PROC UNIVARIATE in SAS 9.4 (SAS Institute, Inc., Cary, NC) and were found to be normal. There were no treatment by year interactions, so data were combined for analysis. Yield data were subjected to ANOVA using the PROC GLIMMIX procedure in SAS 9.4. No differences were found between treatments within annual ryegrass variety, so yield data were pooled within annual ryegrass variety and compared across variety using contrast statements using the PROC GLIMMIX procedure.

6.4 Results and Discussion

No symptoms of infection from *C. nebraskensis* were observed on any annual ryegrass variety or the susceptible corn hybrid in either experiment (*data not shown*). In the absence of disease, there were no differences in corn yield between inoculation timings both within and pooled across annual ryegrass variety (*Data not shown*). There were differences in yield for corn planted in the three different annual ryegrass varieties. Corn had the greatest yield when planted into Winter Hawk (7301 kg ha^{-1}), followed by Gulf (6262 kg ha^{-1}), with the lowest yield when planted into Bruiser (5394 kg ha^{-1}) (Table 6.1). In the absence of disease, these yield data suggest there may be a difference in the competitiveness of the annual ryegrass varieties. At termination, the average height of Bruiser was 35 cm, Gulf was 23 cm, and Winter Hawk was 27 cm. Page et al. (2010) found that early vegetative corn growth is affected by shade competition from living plants. They reported corn was taller, with fewer leaves, and reduced biomass when corn was subjected to shade competition compared to weed-free plots. Weeds were then removed from all plots, yet physiological changes persisted into reproductive corn stages. The authors reported delayed silking and reduced leaf and ear biomass for corn plants exposed to early season shade compared to weed-free plots. The differences in annual ryegrass variety height in our study could have contributed to differences in corn yield loss due to increased shade with taller ryegrass plants. We did not measure stand density or annual ryegrass biomass, so other components of plant competition like water or nutrient competition, cannot be speculated upon (Harper 1977).

6.5 Conclusions

Greenhouse studies have shown that annual ryegrass is a weaker host of *C. nebraskensis* than corn (Campbell 2017; Ikley et al. 2015). Greenhouse temperatures in those studies ranged from 21 to 31 C throughout the experiments. Optimal growth of *C. nebraskensis* occurs at 27 C (Smidt and Vidaver 1986). Average monthly temperature only exceeded 20 C in June 2015 and June 2016 in our experiments (Table 6.2). Annual ryegrass was terminated in early June of both years, so there was little overlap of actively growing annual ryegrass with favorable temperatures for colonization and growth of *C. nebraskensis*. Mallowa et al. (2016) reported that *C. nebraskensis* growth is more favorable in high-humidity environments. Humidity in Indiana is generally higher in the summer compared to the other seasons. The inoculum concentration used in this study (1 x 10^6 CFU per ml) has been sufficient to cause symptoms on annual ryegrass in the greenhouse (Campbell 2017). This indicates the environment may have played the largest role in failure of *C. nebraskensis* to cause symptoms in annual ryegrass. Multiple studies have reported epiphytic populations of *C. nebraskensis* on corn throughout the summer months that eventually led to infection (Eggenberger et al 2016; Mallowa et al. 2016; Smidt and Vidaver 1986). The low temperatures and humidity during all inoculation timings in our study may have inhibited the ability of *C. nebraskensis* to survive epiphytically on annual ryegrass until more favorable environmental conditions arrived. Based on weather conditions during our experiments, it appears that temperature and humidity in Indiana are generally too low to support annual ryegrass infection of *C. nebraskensis* in the field.

We tested three different annual ryegrass varieties in this study to see if there was any difference in disease response throughout their life cycle. It is well established that different corn hybrids respond differently to *C. nebraskensis* infection (Calub et al. 1974a;

Mbofung et al. 2017). Since we did not detect any disease throughout both years, a greenhouse screen would be more effective to determine any differences between annual ryegrass varieties or inoculation timing. This could have more benefit in the southern US where temperatures are generally warmer than Indiana, and annual ryegrass is used as a forage crop in addition to its use as a cover crop. Due to the importance of annual ryegrass as a forage crop, many varieties are rated for tolerance to important diseases of cool season grasses (Braverman 1986). With Goss's wilt has being reported as far south as Texas and Louisiana (Korus et al. 2011; Singh et al. 2015), determining varietal differences or the ability of *C. nebraskensis* to cause disease in annual ryegrass in the field in those states could glean valuable information. Based on our results, it does not appear that *C. nebraskensis* can infect annual ryegrass in the field during its life cycle, and thus annual ryegrass control is not as important as other cultural practices for Goss's wilt management.

6.6 Literature Cited

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Table 6.1. Influence of annual ryegrass variety on corn yields in 2015 and 2016. Data are pooled across inoculation timing within annual ryegrass variety and analyzed using contrast statements.

Variety	Yield	Variety	Yield	*P*-value
	Kg ha^{-1}		Kg ha^{-1}	
Winter Hawk	7301	Bruiser	5394	<0.0001
Winter Hawk	7301	Gulf	6262	0.0122
Gulf	6262	Bruiser	5394	0.0036

Table 6.2. Average daily air temperature and cumulative precipitation for each month during annual ryegrass growth for both experiments at the Agronomy Center for Research and Education near West Lafayette, IN in 2014 through 2016.

Month	Experiment one Fall 2014 to spring 2015		Experiment two Fall 2015 to spring 2016	
	Temp[a]	Precip	Temp	Precip
	C	cm	C	cm
September	17	12.85	20	7.80
October	12	9.96	13	2.41
November	1	3.46	8	5.33
December	1	5.96	5	20.90
January	-4	5.29	-3	2.91
February	-7	2.96	0	3.46
March	2	6.02	8	13.10
April	11	5.64	11	6.66
May	18	8.81	16	4.34
June	21	19.86	23	11.94

[a]Abbreviations: Temp = temperature; Precip = precipitation.

Figure 6.1. Inoculation device used at annual ryegrass bolting and seedhead emergence inoculation timing. The pipe was pulled by a rope which allowed thumb tacks to roll over annual ryegrass plants to cause wounds.

VITA

Joseph Thomas Ikley

Education

Ph.D. **Purdue University**
Weed Science – 2018 (anticipated)
Dissertation: **The Role of Alternative Hosts and Herbicides in the Management of *Clavibacter nebraskensis*, Causal Agent of Goss's wilt of Corn**

M.S. **University of Maryland, College Park**
Plant Science – 2012
Thesis: **The utility of saflufenacil on glyphosate-resistant horseweed and its effect on select soybean varieties**

B.S. **University of Maryland, College Park**
Agricultural Sciences and Technology – 2010

Professional Experience

Purdue University

Weed Science Program Specialist – 2017 to present

- Present Purdue Weed Science research to Indiana stakeholders including farmers, crop consultants, pesticide applicators, and industry personnel
- Develop extension materials for Purdue Weed Science Group
- Part of Purdue Diagnostic Training Center advisory committee: aid planning, establishing, and maintaining weed demonstration plots
- Primary Weed Science consultant for the Purdue Plant and Pest Diagnostic Laboratory. Responsibilities include weed identification, herbicide injury diagnosis, and aiding in diagnosing off target movement complaints. Over 300 samples diagnosed.

Research Associate – 2013 to 2017

- In charge of approximately 200 corn, soybean, and wheat herbicide efficacy trials across 6 locations in Indiana.
- Assisted with the training of 7 graduate students and helped them conduct approximately 40 projects related to their degrees.
- Trained and supervised 28 undergraduate student workers to help assist the Weed Science research program and graduate students complete field, lab, and greenhouse research trials.

Ph.D. Candidate – 2012 to present

- Conducted greenhouse trial to determine host range of the bacterium *Clavibacter michiganensis* subsp. *nebraskensis* (Cmn), causal agent of Goss's wilt of corn.
- Investigated the overwintering capabilities of Cmn on corn and weed hosts debris in Indiana and the ability to become seed-borne in alternative hosts. Studies also researched the ability to overwinter on a cover crop host and transfer to a subsequent corn crop.
- Investigated the role of herbicides in epidemiology of Goss's wilt. This includes testing the survival and pathogenicity of Cmn recovered from infected weeds that were treated with herbicides, the efficacy of herbicides on weeds that were infected with Cmn vs. those that were disease free, and the response in disease severity of Cmn-infected corn after postemergence treatment of herbicides.
- Assisted with the training of one M.S. student who conducted similar research for his thesis. I helped train the student in field, greenhouse, and lab techniques to conduct the research and assisted with trial design and manuscript reviews.

University of Maryland, College Park

Graduate Research Assistant – 2010 to 2012

- Acted as primary research technician and assisted with approximately 100 corn, soybean, small grains, alfalfa, pasture, and sunflower herbicide efficacy trials across 4 sites.
- Conducted field and greenhouse research investigating the utility of saflufenacil for controlling glyphosate-resistant horseweed in no-till soybean.

Undergraduate Research Assistant – 2008 to 2010

- Acted as primary research technician and assisted with approximately 150 corn, soybean, small grains, alfalfa, pasture, and sunflower herbicide efficacy trials across 4 sites.

Teaching Experience

Purdue University

Teaching Assistant, Introductory Weed Science – 2016

- Assisted with preparing and teaching weekly laboratory exercises, including weed identification, sprayer calibration, herbicide identification, and herbicide properties.
- Lead teaching assistant for herbicide calibration, sprayer calibration, and postemergence herbicide activity labs.
- Collaborated with fellow TA's in grading weed collections, lab reports, and quizzes.

University of Maryland, College Park

Teaching Assistant, Introductory Crop Science – 2010

- Assisted with preparing lab materials and teaching labs focused on crop and weed identification and management and sprayer calibration. Graded lab reports and quizzes.

Publications

Peer Reviewed Publications

1. Meyer CJ, Norsworthy JK, Young BG, Steckel LE, Bradley KW, Johnson WG, Loux MM, Davis VM, Kruger GR, Bararpour MT, Ikley JT, Spaunhorst DJ, Butts TR (2016) Early-Season Palmer Amaranth and Waterhemp Control from Preemergence Programs Utilizing 4-Hydroxyphenylpyruvate Dioxygenase–Inhibiting and Auxinic Herbicides in Soybean. Weed Technology 30: 67-75
2. Ikley JT, Wise KA, Johnson WG (2015) Annual Ryegrass (*Lolium multiflorum*), Johnsongrass (*Sorghum haelepense*), and Large Crabgrass (*Digitaria* sanguinalis) are Alternative Hosts for *Clavibacter michiganensis* subsp. *nebraskensis*, Causal Agent of Goss's Wilt of Corn. Weed Science 63: 901-909

Talks, Abstracts, and Other Professional Papers

1. Ikley, J. T., W. G. Johnson, and B. G. Young. 2017. Demonstrating Herbicide Programs in Current and Future Soybean Technologies. NCWSS 72:46.
2. Campbell, T. C., J. T. Ikley, and W. G. Johnson. 2017. Influence of Fall Establishment and Spring Termination Timings of Annual Ryegrass on Corn Yields. NCWSS 72:69.
3. Ikley, J. T. and W. G. Johnson. 2017. Launching Roundup ready Xtend Soybean in a Wet and Windy Year: Perspectives from Indiana. NCWSS 72:209.
4. Ikley, J. T., K. A. Wise, and W. G. Johnson. 2016. Effect of Single Mode of Action Postemergence Herbicides on Severity of Goss's wilt in Corn. NCWSS 71:149.
5. Campbell, T. C., J. T. Ikley, K. A. Wise, and W. G. Johnson. 2016. Response of Three Alternative Weed Hosts and Two Corn Hybrids to Different Inoculum Levels of *Clavibacter michiganensis* subsp. *nebraskensis*, Causal Agent of Goss's wilt. NCWSS 71:133.
6. Campbell, T. C., J. T. Ikley, K. A. Wise, and W. G. Johnson. 2016. Survey of Field Practices, Environmental Factors, and Alternative Weed Hosts Influencing the Occurrence of Goss's Wilt. NCWSS 71:98.
7. Ikley, J. T., K. A. Wise, and W. G. Johnson. 2016. Ability of *Clavibacter michiganensis* subsp. *nebraskensis*, Causal Agent of Goss's wilt of Corn, to Overwinter in Alternative Host Debris and Seed. NCWSS 71:97.

8. Campbell, T. C., J. T. Ikley, K. A. Wise, and W. G. Johnson. 2015. Alternative Hosts Response to Inoculum Levels of *Clavibacter michiganensis* subsp. *nebraskensis*. NCWSS 70:132.
9. Ikley, J. T., K. A. Wise, and W. G. Johnson. 2015. Ability of *Clavibacter michiganensis* subsp. *nebraskensis*, Causal Agent of Goss's wilt of Corn, to Overwinter on Alternative Hosts. NCWSS 70:131.
10. Ikley, J. T., K. A. Wise, and W. G. Johnson. 2015. Effect of Postemergence Herbicides on Disease Severity of Goss's wilt in Corn. NCWSS 70:91.
11. Campbell, T. C., J. T. Ikley, K. A. Wise, and W. G. Johnson. 2015. Survey of Alternative Hosts and Cultural Practices in Indiana Fields Infected with Goss's Wilt. NCWSS 70:77.
12. Ikley, J. T., W. G. Johnson, and K. A. Wise. 2015. Defining the alternative host range and the effect of herbicides on pathogenicity of *Clavibacter michiganensis* subsp. *nebraskensis*, causal agent of Goss's wilt of corn. Phytopatohlogy 106 (1S): S1.4.
13. Campbell, T. C., J. T. Ikley, K. A. Wise, and W. G. Johnson. 2014. Effect of Inoculum Concentration of *Clavibacter michiganensis* subsp. *nebraskensis* on Infection of Alternative Hosts. NCWSS 69:126.
14. Ikley, J. T., T. R. Legleiter, and W. G. Johnson. 2014. Comparison of Herbicide Mode of Action Combinations Applied Preemergence to Glyphosate-Resistant Palmer Amaranth in Indiana. NCWSS 69:104.
15. Ikley, J. T., T. R. Legleiter, and W. G. Johnson. 2014. Comparison of Single Mode of Action Herbicides Applied Preemergence to Glyphosate-Resistant Palmer Amaranth in Indiana. NCWSS 69:103.
16. Ikley, J. T., K. A. Wise, and W. G. Johnson. 2014. Effect of Herbicides on Recovery and Pathogenicity of *Clavibacter michiganensis* subsp. *nebraskensis*, Causal Agent of Goss's Wilt of Corn. NCWSS 69:74.
17. Ikley, J. T., K. A. Wise, and W. G. Johnson. 2013. - Impact of Herbicides on *Clavibacter michiganensis* subsp. *nebraskensis*, Causal Agent of Goss's Wilt of Corn. NCWSS 68:118.
18. Ikley, J. T., K. A. Wise, and W. G. Johnson. 2013. Defining the Weed Host Range of *Clavibacter michiganensis* subsp. *nebraskensis*, Causal Agent of Goss's Wilt of Corn. NCWSS 68:79.
19. Ikley, J. T., K. A. Wise, and W. G. Johnson. 2012. Weed Hosts of *Clavibacter michiganensis* subsp. *nebraskensis*, causal agent of Goss's bacterial wilt and leaf blight. NCWSS 67:103.
20. Ikley, J. and R. L. Ritter. 2012. Effect of saflufenacil application timing on soybean and its role in managing glyphosate-resistant horseweed (*Conyza canadensis*). WSSA 52:194.
21. Ikley, J. and R. L. Ritter. 2012. Effect of saflufenacil application timing on soybean and its role in managing glyphosate-resistant horseweed. NEWSS 66:38.
22. Ritter, R. L. and J. Ikley. 2011. Pyroxasulfone: It's fit in row crops for the Mid-Atlantic region. NEWSS 65:92.
23. Ritter, R. L. and J. Ikley. 2010. New tools for the management of glyphosate-tolerant horseweed in full-season no-till soybeans. NEWSS 64:117.

24. Ritter, R. L., H. Menbere and J. Ikley. 2009. Management of Italian ryegrass (*Lolium multiflorum*) in wheat. WSSA 49:344.
25. Ritter, R. L., H. Menbere and J. Ikley. 2009. Italian ryegrass control in wheat. NEWSS 63:52.

Reports – Annual Results of Weed Control Research

1. Ritter, R. L., M. Morris, and J. Ikley. 2012. 374pp.
2. Ritter, R. L. and J. Ikley. 2011. 336pp.
3. Ritter, R. L. and J. Ikley. 2010. 232pp.
4. Ritter, R. L. and J. Ikley. 2009. 374pp.
5. Ritter, R. L., H. Menbere, and J. Ikley. 2008. 308pp.

Extension and Outreach

Extension Publications

Peer Reviewed Extension Publications

1. Johnson, B., J. Ikley, A. Hager, and M. Loux. 2017. Precautions for Dicamba Use in Xtend Soybeans (WS-55). Published 01/2018.
2. Freije, A., J. Ikley, K. Wise, and B. Johnson. 2015. Goss's Wilt on Grass Hosts (BP-88-W). Published 9/22/2015.

Purdue Extension Publications

1. Corn and Soybean field Guide (ID-179). 2018. Annual updated pocket guide designed and distributed through the Purdue Diagnostic and Training Center. Contributing author to Weed ID and Herbicide Symptomology sections. Approx. 40,000 – 50,000 copies distributed annually.

Regional Extension Publications

1. Loux. M. M., D. Doohan, A. F. Dobbels, B. Reeb (The Ohio State University); W. G. Johnson, B. G. Young, J. Ikley (Purdue University); and A. Hager (University of Illinois). 2018. Ohio, Indiana, and Illinois Weed Control guide (WS16). Approx. 6,000 copies distributed annually to agricultural producers in Indiana, Illinois, and Ohio.
2. Johnson, B., J. Ikley (Purdue University); and M. Loux (The Ohio State University). 2017. A Quick Guide to Using LibertyLink Soybeans Effectively.

Extension Newsletters

- Authored or co-authored 18 articles published in the 2017 edition of Purdue's Pest and Crop Newsletter, the primary Purdue Extension resource for row crop management topics that reaches over 3,200 individuals weekly.

Extension Presentations

Invited Presentations

1. Threading the Needle: A Weed Scientist's Perspective. Presented at the 71st Association of American Pesticide Control Official Conference on March 6th 2018. 150 attendees.
2. Perspectives on Dicamba in the Eastern Corn Belt. Presented at the South Dakota Agribusiness Agronomy Conference on December 13th 2017. 250 attendees.
3. Launching Xtend Soybean in Indiana: Fallout from a Wet and Windy Year. Presented at the Indiana Certified Crop Adviser Conference on December 12th 2017. 372 attendees.
4. Purdue Perspectives on New Soybean Technologies. Presented to Asociacion de Cooperativas Argentinas on October 6th 2017. 8 attendees.
5. Dicamba Update. Presented at the 151st Indiana Pesticide Review Board Meeting on August 30th 2017. 65 attendees.

Train the Trainer

- Mandatory Dicamba Training. Worked with the Purdue Pesticide Program and Office of Indiana State Chemist to develop and implement mandatory dicamba training for Indiana applicators to fulfill federal training requirements. Trained 70 approved educators at 1 in person event and 2 webinars.

Purdue Crop Management Workshop

- Annual workshop hosted by Purdue Integrated Pest Management that is attended primarily by certified crop advisors and industry field staff.
    1. Presented at 3 workshops to approximately 600 attendees in 2018
    2. Presented at 3 workshops to approximately 550 attendees in 2017.

Diagnostic Training Center and Regional Field Days

- Hands on training conducted at the Purdue Diagnostic Training Center (DTC) and seasonal updates at regional Purdue Agricultural Center (PAC) Field Days. Subjects include weed identification, weed control, herbicide symptomology, and spray technology. Presented at :
    1. Eleven DTC training events to 400 people in 2017.
    2. Four PAC field days to 1,200 people in 2017.
- Dow New Hire Training Workshop. Collaborated with Corey Gerber, director of the Diagnostic Training Center, to teach sprayer calibration new Dow employees. Trained 50 employees in 2017.
- Indiana Association of Soil and Water Conservation District (IASWCD) Weed Identification Training. Taught members of the IASWCD and Purdue Extension half-day programs covering weed identification using identification keys. Additional topics included weed science updates and general weed control principles. Presented at:
    1. One webinar to 160 people in 2018.

2. Two trainings to 120 people in 2017.

Indiana Category I Applicator Training

- Training workshop for potential pesticide applicators. Hosted by the Purdue Pesticides Program. Weed Management Principles taught to approximately 75 attendees at 1 workshop in 2015.

Pesticide Applicator Recertification Programs

- Pesticide Applicator Recertification Programs (PARP) are hosted throughout the state of Indiana by Purdue Extension educators for continuing education and credits for private pesticide applicators. A variety of weed science topics relevant to Indiana farmers and applicators have been presented at:
    1. Eighteen programs in 2018 to approximately 1,600 people
    2. Fourteen programs in 2017 to approximately 750 people
    3. Three programs in 2016 to approximately 150 people
    4. Two programs in 2015 to approximately 100 people
    5. Two programs in 2014 to approximately 300 people

Professional Memberships

Weed Science Society of America – 2010 to present
Northeast Weed Science Society – 2009 to 2013
Member, Photo Judging Committee – 2011 to 2013
North Central Weed Science Society – 2012 to present
Chair, Agronomic Crops Section – 2015
American Phytopathological Society – 2013 to present
Purdue University Cooperative Extension Specialist Association (PUCESA)
Botany and Plant Pathology Representative – 2017 to present
Nomination Committee – 2018 to present

Awards & Honors

North Central Weed Science Society

- Outstanding Graduate Student, Finalist – 2017
- Outstanding Graduate Student Paper Award, 1st Place in Agronomic Crops I – 2016
- Weed Contest – Third Place Overall Graduate Team – 2015
- Outstanding Graduate Student Poster Award, 1st Place in Herbicide Efficacy – 2014

North Central American Phytopathological Society

- Student Travel Award – 2015

Undergraduate Scholarships

- The Delmarva Farmer Scholarship – 2009
- Joseph Newcomer Memorial Scholarship – 2009
- Maryland Crop Improvement Association Scholarship – 2009