Investigation of Alternative Hosts and Agronomic Factors Affecting *Xanthomonas vasicola* pv. *vasculorum*, Causal Agent of Bacterial Leaf Streak of Corn

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INVESTIGATION OF ALTERNATIVE HOSTS AND AGRONOMIC FACTORS AFFECTING \textit{Xanthomonas vasicola} pv. \textit{Vascularorum}, CAUSAL AGENT OF BACTERIAL LEAF STREAK OF CORN

By

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INVESTIGATION OF ALTERNATIVE HOSTS AND AGRONOMIC FACTORS
AFFECTING *XANTHOMONAS VASICOLA* PV. *VASCULORUM*, CAUSAL AGENT
OF BACTERIAL LEAF STREAK OF CORN

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University of Nebraska, 2018

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*Xanthomonas vasicola* pv. *vasculorum*, causal agent of bacterial leaf streak of corn, was first reported in the U.S. in 2016 on Nebraska corn leaf samples. Prior to this report, the pathogen had only been reported on corn in South Africa. After the Nebraska report, the pathogen was reported on corn in Argentina, Brazil, and several U.S. states. This pathogen has an extensive host range, which includes sugarcane (*Saccharum officinarum*), grain sorghum (*Sorghum bicolor*), palm species (*Dictyosperma album*, *Roystonea regia*, and *Areca catechu*) and broom bamboo (*Thysanolaena maxima*). Plants commonly found in the U.S. had not been evaluated as alternative hosts. Additionally, the distribution of *X. vasicola* pv. *vasculorum* in Nebraska and agronomic factors that influence its survival had not been investigated. A survey was conducted to determine the pathogen’s distribution, and to better understand agronomic factors that may influence disease. Respondents submitted a symptomatic leaf sample for pathogen confirmation with their completed survey. The pathogen was confirmed in 74 Nebraska counties via a PCR assay. Survey results indicated that the strongest predictors of a sample testing positive for *X. vasicola* pv. *vasculorum* included irrigation, planting date, crop rotation, tillage, and growth stage. A greenhouse experiment was conducted to
examine U.S. plant species that could serve as alternative hosts for the pathogen. Hosts included oat (*Avena sativa*), rice (*Oryza sativa*), orchardgrass (*Dactylis glomerata*), indiangrass (*Sorghastrum nutans*), big bluestem (*Andropogon gerardii*), little bluestem (*Schizachyrium scoparium*), timothy (*Phleum pratense*), sand bluestem (*Andropogon hallii*), green foxtail (*Setaria viridis*), bristly foxtail (*Setaria verticillata*), johnsongrass (*Sorghum halepense*), shattercane (*Sorghum bicolor*), yellow nutsedge (*Cyperus esculentus*), downy brome (*Bromus tectorum*), tall fescue (*Festuca arundinacea*), and western wheatgrass (*Pascopyrum smithii*). A field study examined the potential for these plants to become infected by the pathogen in a natural environment. Host species were transplanted to a field naturally infested with *X. vasicola pv. vasculorum*. With natural inoculum, big bluestem and bristly foxtail displayed symptoms, and the pathogen was isolated from them. These results indicate that certain agronomic practices, as well as alternative hosts found in and near corn fields, may influence the survival of *X. vasicola pv. vasculorum*. 
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CHAPTER I

LITERATURE REVIEW
Literature Review

Background of Corn

Corn (*Zea mays* L.), or maize as it’s known in other parts of the world, is a monocotyledonous, annual crop that emerged as a result of domestication of species of teosinte. Through selection, teosinte was modified to have larger ears, more starchy kernels, and thicker stalks (Beadle 1980). Despite these differences, there are only five genomic regions that distinguish corn and teosinte, and the two can still interbreed with each other and produce viable offspring (Doebly and Stec 1991). Modern day corn was first cultivated by natives of Mexico 7,000 years ago, but is now distributed worldwide (Beadle 1980). When Christopher Columbus landed in Cuba in 1492, the natives introduced Columbus to corn, which he brought back to Europe. After corn was introduced to Europe, it spread to most of the eastern hemisphere within 100 years (Smith 2004).

From 1866 to 1930, there was almost no change in the average yield of corn, but there was a rapid increase in average yield from 1950 to 2005, as high input agriculture, especially the use of nitrogenous fertilizers, became more common (Egli 2006). Today, corn is a very important crop to Nebraska and to the United States. Corn is grown in all 50 states, with the top five corn producers by bushel being Iowa, Illinois, Nebraska, Minnesota, and Indiana, respectively. At 9,186,360,000 bushels in 2016, these states produced 60.64% of the corn crop in the United States (USDA, NASS Crop Production Summary, 2016). One limiting factor to corn production may be a newly introduced pathogen. Since its initial discovery in the United States in 2014, the causative agent of
bacterial leaf streak in corn, *Xanthomonas vasicola pv. vasculorum*, has been confirmed and reported in four of the top five corn producing states, with Indiana being the only exception (Korus et al. 2017; Bowman et al. 2016).

**Xanthomonas vasicola pv. vasculorum Symptoms and Distribution**

Bacterial leaf streak disease of corn is caused by a gram-negative, rod-shaped bacterium known as *X. vasicola pv. vasculorum* (Dyer 1949). This pathogen is most noted for causing gumming disease of sugarcane (*Saccharum* spp.). Gumming disease symptoms include a leaf blight phase and a vascular wilt phase. In the leaf blight phase, dark to light brown streaks form on the leaves. These streaks can coalesce to form larger necrotic areas on the leaf. If the disease becomes systemic, the bacteria can block the vascular system of the plant, causing a systemic wilt, hence the vascular wilt phase, and potentially whole plant death (North 1935). Gumming disease is distributed in sugarcane growing regions throughout the world, and was reported prior to 2014 in Europe, South Africa, East Asia, South America, and Mexico. Gumming disease has not been reported in sugarcane grown in the continental United States (CAB International 1999). When *X. vasicola pv. vasculorum* was first reported in sugarcane in Natal, researchers speculated that the pathogen had been present for years before being reported. The disease was only noticed when a particularly susceptible variety of sugarcane was introduced and widely grown. This coupled with favorable weather conditions for disease development led to a serious gumming disease outbreak in Natal in 1956 (Thompson, 1957).

Bacterial leaf streak symptoms on corn include yellow, tan, or dark brown, interveinal streaks on the leaves. These streaks can be long or short, and distributed
throughout the leaf blade or concentrated around the midrib. Streaks (also known as lesions) typically have a wavy margin, rather than a rectangular shape. In the early stages of infection, the streaks have a water-soaked or yellow appearance before becoming dark to light brown color. The streaks typically have yellow halos when backlit (Figure 1). In particularly humid environments, the pathogen may also produce a yellow exudate comprised of xanthomonadin and extracellular polysaccharide on the surface of the leaves (Korus et al. 2017; Chun et al. 1997).

**History of Bacterial Leaf Streak in Corn**

Bacterial leaf streak of corn, caused by *X. vasicola pv. vasculorum*, was first reported in South Africa in 1949 (Dyer 1949). It had only been reported on corn in South Africa until 2016, when it was first reported on corn in the United States. The first finding of suspect bacterial leaf streak symptoms in the United States occurred on Nebraska corn leaf samples in 2014. These symptoms were observed on samples from 41 Nebraska counties, one county in Colorado, and one county in Kansas. The pathogen was isolated, confirmed via testing of Koch’s Postulates, and identified as *X. vasicola pv. vasculorum* in 2015. By 2018, the disease had been confirmed in Colorado, Illinois, Iowa, Kansas, Nebraska, Oklahoma, South Dakota, Texas, and Wisconsin (Korus et al. 2017; Bowman et al. 2016; Smith et al. 2018). Since the initial report in the United States, the pathogen has also been reported in Argentina (Plazas et al. 2018) and Brazil (Augusto et al. 2018). It is currently unclear how the pathogen was introduced into the United States.
Taxonomic Classification of *Xanthomonas vasicola pv. vasculorum*

The causative agent of bacterial leaf streak in corn and gumming disease in sugarcane has been taxonomically reclassified multiple times. This is partially due to the methods of examining species relatedness at the genomic level becoming more sophisticated. The pathogen was first named *X. campestris pv. vasculorum* (Bradbury 1986), and this designation included strains from both sugarcane and corn in South Africa. The corn pathogen was later renamed *X. campestris pv. zeae*, while the name of the sugarcane pathogen remained the same. This differentiation was based on DNA restriction patterns, and on the different hosts and disease symptoms, specifically the vascular wilt phase, which occurs in sugarcane but not in corn (Qhobela and Claflin 1992).

More recent research has indicated that both the corn and the sugarcane pathogen should be classified as *X. vasicola pv. vasculorum* based on genomic comparisons and pathogenicity tests (Wasukira et al. 2014; Karamura et al. 2015; Lang et al. 2017). In average nucleotide identity calculations, the genomes of the pathogen causing bacterial leaf streak in Nebraska corn were found to be greater than 99.3% identical to the genomes of the *X. vasicola pv. vasculorum* strains from corn and sugarcane in South Africa. Results of pathogenicity tests showed that all strains, regardless of source, were virulent on both corn and sugarcane (Lang et al. 2017).

A closely related pathogen is *Xanthomonas vasicola pv. holicicola*. This xanthomonad has only been observed causing disease on sorghum in the field, however strains of this pathogen also caused disease on both corn and sugarcane in pathogenicity
tests by Lang et al. (2017). Although *X. vasicola* pv. *holcicola* caused disease on all tested hosts, this pathogen was most virulent on sorghum, and was not as virulent on corn or sugarcane as *X. vasicola* pv. *vasculorum*. Average nucleotide identity values calculated in this same study indicated that *X. vasicola* pv. *holcicola* and *X. vasicola* strains obtained from Nebraska corn were 98.6% identical to *X. vasicola* pv. *holcicola*. The lower genetic similarity, as well as the differences in pathogenicity, indicated that the xanthomonad isolated from Nebraska corn was *X. vasicola* pv. *vasculorum*, and not *X. vasicola* pv. *holcicola*.

**Variation in Xanthomonas vasicola pv. vasculorum strains**

Since the report by North (1935), there have been documented genotypic and phenotypic differences among strains of *X. vasicola* pv. *vasculorum* isolated around the world. Based on phenotypic differences, it was suspected that strains of *X. vasicola* pv. *vasculorum* from East Africa were different from strains from South Africa, although they caused the same disease. Qhobela and Claflin (1992) demonstrated through restriction fragment length polymorphism (rflp) that these strains were distinct. In a separate experiment, Qhobela and Claflin (1992) found that there were differences in strain membrane proteins. Dookun et al. (2000) further examined variation in *X. vasicola* pv. *vasculorum* with fatty acid profiling. They demonstrated that *X. vasicola* pv. *vasculorum* strains should be divided into five groups. All Group A strains were obtained from broom bamboo (*Thysanolaena maxima*) in Mauritius, East Africa. Group B strains were obtained from palms (Palmae), broom bamboo, and sugarcane from Mauritius, Réunion and Australia. Group C strains were obtained from corn and sugarcane in South
Africa and Malagasy. Group D strains were obtained from sugarcane, corn, and royal palm (*Roystonea regia*) in Mauritius and Réunion. Only one *X. vasicola* pv. *vasculorum* strain was placed in Group E, a strain obtained from sugarcane in Puerto Rico. Among strains from Mauritius, *X. vasicola* pv. *vasculorum* strain morphology varied to such an extent that it was suspected that symptoms were caused by different races of the pathogen. However, Peros demonstrated in 1988 that these differences were due to strain aggressiveness. Strains obtained from United States corn have not been grouped in this manner.

Although they are the same pathogen, strains of *X. vasicola* pv. *vasculorum* isolated from corn differ somewhat from strains isolated from sugarcane. According to Lang et al. (2017), one notable difference between strains isolated from corn and strains isolated from sugarcane is the *xopAF* effector gene, which was present in all tested sugarcane strains but no corn strains.

**Epidemiology and Survival of Xanthomonas vasicola pv. vasculorum and Related Pathogens**

While there is no quantitative data on optimum temperatures for the growth and survival of *X. vasicola* pv. *vasculorum*, according to Koizumi (1985) another xanthomonad, *Xanthomonas axonopodis* pv. *citri*, can function at 6°C-36°C, although temperatures of 15°C-35°C were optimal for infection and subsequent symptom development (Peltier, 1920). Given that this temperature range often occurs during the corn growing season in Nebraska, it is possible that *X. vasicola* pv. *vasculorum* is also able to survive in these temperatures. *X. vasicola* pv. *vasculorum* is capable of infecting
through natural openings in the plant, such as the stomata, which are kept open longer as humidity levels increase. A high-moisture, high humidity environment is often present in Nebraska cornfields, which frequently use center pivot irrigation (Turkington et al. 2004; Lichtenberg 1989). The pathogen is also capable of penetrating wounds on the plant if they are present (Kevin Korus, personal communication). Early season symptoms often begin on the bottom leaves of the plant and move up the plant throughout the corn growing season, while mid-season symptoms may be observed beginning in the mid to upper leaves. This is especially true after a wind-driven rain event (Tamra Jackson-Ziems, personal communication). Other xanthomonads, such as *X. axonopodis pv. allii*, causal agent of Xanthomonas leaf blight of onion, have been shown to be residue-borne (Gent et al. 2005). This is also thought to be true of *X. vasicola pv. vasculorum* due to the distribution and movement of the symptoms on the plant (Hartman et al. 2017). Given this distribution and movement, it is also possible that infested soil is a source of *X. vasicola pv. vasculorum* inoculum, which is true of *Xanthomonas campestris pv. vesicatoria* (O’Garro et al. 1997).

Wind-driven rain dispenses inoculum and exacerbates diseases caused by other xanthomonads, including Xanthomonas leaf blight of onion (Schwartz et al. 2003). This has been observed in bacterial leaf streak of corn as well, however as of now no study has been conducted to formally investigate the extent to which wind-driven rain affects bacterial leaf streak development. Disease development in sugarcane is most severe in cold or dry weather and waterlogged soils. These conditions tend to allow the systemic phase of the disease to occur in sugarcane (North et al. 1935), however these
environmental stressors could have a different impact on disease development in corn due
to host differences and pathogen strain differences. According to a study on gumming
disease by Coutinho et al. (2015), *X. vasicola pv. vasculorum* can be spread by insects
when they land in bacterial exudate on leaves and then move to a non-infected plant, but
this has not been examined in bacterial leaf streak of corn. The likelihood of insects being
a significant mechanism of dispersal is partially dependent on the environment, which
influences the extent to which the bacterium will exude on the leaf surface (Coutinho et
al. 2015). *X. axonopodis pv. allii* of onion can survive in rain and irrigation water, and
can be spread from field to field in runoff (Gent et al. 2005), but this has not yet been
examined with *X. vasicola pv. vasculorum*. Several other xanthomonads, such as *X.
campestris pv. vesicatoria*, *X. campestris pv. phaseoli* (Moffet and Croft 1983), and *X.
campestris pv. glycines* (Singh and Jain 1988) are seed-borne, and it is possible that *X.
vasicola pv. vasculorum* is also seed-borne, albeit at low levels (Arias et al. 2017).

The known host range of *X. vasicola pv. vasculorum* is very wide, and includes
corn, sugarcane, grain sorghum, palm (*Dictyosperma album, Roystonea regia, and Areca
catechu*), and broom bamboo (*Thysanolaena maxima*) (Ricaud and Autrey, 1989;
Dookun et al. 2000; Qhobela et al. 1990; Lang et al. 2017). This wide host range is likely
due to the plasticity of the genome of xanthomonads, which makes the bacterium highly
adaptable to different hosts and environments (Thieme et al. 2005).

Xanthomonadin, a yellow, brominated pigment produced exclusively by members
of the *Xanthomonas* genus (Andrewes et al. 1973), protects xanthomonads from
photodegradation before they enter the plant (Jenkins and Starr 1982). Poplowsky and
Chun (1995) demonstrated that the extracellular polysaccharide layer exuded by xanthomonads protects *Xanthomonas campestris* pv. *campestris* from harsh environmental factors as the pathogen exists epiphytically on crucifer leaves. Mutant strains of *X. campestris* pv. *campestris* deficient in xanthomonadin and extracellular polysaccharide production colonize plants at significantly lower population densities than wild type strains (Chun et al. 1997). These compounds are essential for pathogen survival, and they allow it to survive epiphytically on host plants in environments that are non-conducive to pathogen growth and disease development.

Research by Timmer et al. (1987) also indicated that *Xanthomonas campestris* pv. *citrumelo* can multiply epiphytically on host plants before and during infection and subsequent symptom development. This is especially true when conditions are favorable for the survival of xanthomonads, such as in a moist, humid environment. Populations of xanthomonads on the leaf surface remain high in such environments. In an environment that is non-conducive to the survival of xanthomonads, populations will decrease over time, but there will likely be some individuals that will survive (Timmer et al. 1987).

Endophytic survival of *X. vasicola* pv. *vasculorum* has not been investigated, other xanthomonads, however, have been reported to exist as endophytes in their host species. In a study by Velázquez et al. (2008), *Xanthomonas campestris*, a xanthomonad related to *X. vasicola* pv. *vasculorum*, was isolated as an endophyte from asymptomatic sugarcane. This xanthomonad has also been isolated from asymptomatic clover (Burch and Sarathchandra 2006) and citrus leaf tissue (Araujo et al. 2002), demonstrating its ability to inhabit both monocotyledonous and dicotyledonous plants.
Management

Currently, there is little information on the management of this disease in corn. Bactericides have been shown to inhibit the reproduction of xanthomonads in other crops, such as onion (Gent and Schwartz 2005), but will likely not be practical in most corn-growing situations. If bacterial leaf streak is a residue-borne disease, standard management practices for other residue-borne diseases may help to mitigate symptoms. These strategies include crop rotation and tillage, which prevent buildup of infested residue. Equipment sanitation between fields may prevent the dispersal of the pathogen to other fields on equipment (Jackson et al. 2007). However, equipment sanitation to prevent pathogen dispersal has generally been a futile measure when attempting to manage the disease in sugarcane production due to the pathogen’s ability to rapidly and extensively disperse without being moved on equipment (Ricaud and Autrey 1989). Although resistant corn hybrids have not been developed yet, there has been success controlling the pathogen in sugarcane with resistant hybrids (North 1935; Ricaud and Autrey 1989). In sugarcane, genetic resistance to *X. vasicola pv. vasculorum* is not polygenic (Stevenson, 1965), and the genes that determine resistance to the leaf blight symptoms are different from the genes that determine resistance to the vascular wilt (North, 1935; Ricaud, 1969).

Yield Impact

There is currently little information on the potential yield impact of bacterial leaf streak in corn. In sugarcane, a 30-40% reduction in plant tonnage can occur when the systemic phase of infection kills whole plants. Additionally, sugar content is reduced by
9%-17% (North 1935). Research by Ricaud and Autrey et al. (1989) showed that sugarcane plants that developed a systemic *X. vasicola* pv. *vasculorum* infection yielded 19.5% less tonnage than plants that only developed leaf blight symptoms. Due to the differences in usage and processing of corn and sugarcane, and the lack of a vascular wilt phase in corn, these yield losses may not be comparable, however it should be noted that the majority of yield losses in sugarcane occur when the disease reaches the systemic phase.

It is expected that most of the yield impact in corn will be due to loss of photosynthetic tissue as a result of necrotic streaks on the leaves created by the pathogen (Tamra Jackson-Ziems, personal communication). This could have an especially large impact if the pathogen reaches the upper canopy (above the ear leaf). The fungal disease called gray leaf spot (caused by *Cercospora zeae-maydis*) causes interveinal streaks on corn leaves (Hyre 1943), similar in appearance to the symptoms caused by bacterial leaf streak. Gray leaf spot is also a residue-borne disease, therefore symptoms usually begin on the lower leaves and travel up the plant (de Nazareno et al. 1992). Because of the similarity of the symptoms and behavior, the two diseases may have similar effects on yield, however this has not been formally investigated.

The yield impact of gray leaf spot depends on the time at which symptoms begin to develop and the environment. If symptoms develop earlier in the growing season, there is potential for a larger yield impact because the pathogen has more time to spread before environmental conditions become unfavorable for its spread. If the environment is favorable for the development of gray leaf spot then the pathogen infects and spreads
rapidly, causing greater yield impacts (Jenco and Nutter 1992). This is assuming that a fungicide is not applied to control the disease, and it should be noted that hybrid resistance also plays a role in yield impacts. When not controlled with fungicides, gray leaf spot has the potential to reduce yields by up to 65% in susceptible hybrids (Ward et al. 1999). It is possible that bacterial leaf streak could have a similar effect due to the similar symptoms and behavior.

**Summary of Literature Review**

Corn is an important crop in the U.S., and is grown in every state. *X. vasciola pv. vasculum* was recently introduced into the U.S., and is especially prevalent in the states that produce the greatest amount of corn. *X. vasciola pv. vasculum* has an extensive symptomatic host range, and closely related xanthomonads are capable of surviving in asymptomatic plants, however plants commonly found in the U. S. have not been examined as symptomatic or asymptomatic alternative hosts. Chapter II of this thesis will examine plants commonly grown in the U.S. as alternative hosts. While there are speculations on agronomic practices that favor *X. vasciola pv. vasculum* based on anecdotal observations and the behavior of closely related pathogens, there is a lack of data on which factors are associated with the presence of *X. vasciola pv. vasculum*. Chapter III of this thesis will investigate agronomic factors that may be associated with disease.
Literature Cited


Hausbeck, M., Bell, J., Medina-Mora, C., Podolsky, R., Fulbright, D. 2000. Effect of bactericides on population sizes and spread of Clavibacter michiganensis subsp. michiganensis on tomatoes in the greenhouse and on disease development and crop yield in the field. Phytopathology. 90:38-44.


Qhobela, M., Claflin, L.E., Nowell, D.C. 1990. Evidence that *Xanthomonas campestris* pv. *zeae* can be distinguished from other pathovars capable of infecting maize by restriction fragment length polymorphism of genomic DNA. Canadian Journal of Plant Pathology. 12(2):183-186.


Figure 1: Necrotic, coalesced streaks (top left, courtesy of Tamra Jackson-Ziems) and chlorotic streaks (top right) symptomatic of *X. vasicola* pv. *vasculorum* infection on corn, and red streaks with chlorotic halos symptomatic of the leaf blight phase of *X. vasicola* pv. *vasculorum* infection in sugarcane (bottom, from Coutinho et al. 2015).
CHAPTER II

SIXTEEN ALTERNATIVE HOSTS FOR XANTHOMONAS VASICOLA PV.

VASCULORUM, CAUSAL AGENT OF BACTERIAL LEAF STREAK OF CORN,

FROM POACEAE AND CYPERACEAE
Abstract

The bacterial corn pathogen *Xanthomonas vasicola* pv. *vasculorum* was first reported in the United States causing disease on Nebraska corn (*Zea mays*) in 2016. This bacterium is also known for causing disease in sugarcane, broom bamboo, various palm species, and sorghum species. The wide host range indicates that this pathogen may be capable of infecting plants found in the United States. The objective of these experiments was to determine if infection of other plants was possible. Fifty-four species of plants found in the United States were inoculated with a suspension of *X. vasicola* pv. *vasculorum* in water and observed for symptom development. The symptomatic alternative hosts identified included oat (*Avena sativa* ‘Jerry’), rice (*Oryza sativa*), orchardgrass (*Dactylis glomerata* ‘Latar’), indiangrass (*Sorghastrum nutans* ‘Holt’), big bluestem (*Andropogon gerardii* ‘Champ’), little bluestem (*Schizachyrium scoparium* ‘Blaze’), timothy (*Phleum pratense* ‘Climax’), sand bluestem (*Andropogon hallii*), green foxtail (*Setaria viridis*), bristly foxtail (*S. verticillata*), johnsongrass (*Sorghum halepense*), shattercane (*Sorghum bicolor*), and yellow nutsedge (*Cyperus esculentus*). Bacterial colonization was also confirmed in three asymptomatic alternative hosts, including downy brome (*Bromus tectorum*) at $\log_{10} 5.8$ CFU/gram of dry tissue and 60% incidence, tall fescue (*Festuca arundinacea* ‘Cajun II’) at $\log_{10} 5.7$ CFU/gram and 30% incidence, and western wheatgrass (*Pascopyrum smithii*) at $\log_{10} 5.1$ CFU/gram and 60% incidence. To investigate the possibility of infection in the field under natural conditions, species identified as symptomatic hosts were transplanted from the greenhouse into a corn field with a history of bacterial leaf streak in corn and monitored for symptom development.
until senescence. *Andropogon gerardii* Vitman ‘Champ’ and *Setaria verticillata* (L.) Beauv. developed symptoms at 8% and 17% incidence, respectively.

**Introduction**

Corn (*Zea mays*) is the most widely produced grain crop in the United States, and is used primarily in biofuel production and as livestock feed. Iowa produces the greatest amount of corn by bushel, followed by Illinois and Nebraska (USDA, NASS Crop Production Summary, 2016). In 2016, *Xanthomonas vasicola* pv. *vasculorum* was reported on Nebraska corn. This was the first report of the pathogen causing bacterial leaf streak on corn in the United States (Korus et al. 2017). Prior to this report, *X. vasicola* pv. *vasculorum* had only been reported on corn in South Africa (Dyer, 1949). The pathogen has since been confirmed in corn in eight other states, including Colorado, Illinois, Iowa, Kansas, Minnesota, South Dakota, Oklahoma, Texas, and Wisconsin (Korus et al. 2017; Bowman et al. 2017, Smith et al. 2018). Recently, *X. vasicola* pv. *vasculorum* has also been reported on corn in Argentina (Plazas et al. 2018) and Brazil (Augusto et al. 2018). Symptoms include water-soaked, yellow to brown interveinal streaks on the leaves, which are typically first observed in the lower canopy (Korus et al. 2017).

*X. vasicola* pv. *vasculorum* is known to infect several hosts besides corn. It has been reported in sugarcane (*Saccharum* spp.) growing regions around the world, but not in U.S. sugarcane, and is capable of severely damaging a sugarcane crop. In sugarcane, the pathogen causes gumming disease, a leaf blight and vascular wilt (North 1935). According to Ricaud and Autrey (1989), the bacterium also causes leaf blighting of multiple palm species (*Dictyosperma album, Roystonea regia, and Areca catechu*) and
broom bamboo (*Thysanolaena maxima*). Korus et al. (2017) and Lang et al. (2017) demonstrated that *X. vasicola* pv. *vasculorum* can infect grain sorghum (*Sorghum bicolor*), although this has not been observed in the field. Although disease on all of the aforementioned hosts is caused by the same pathogen, one notable genetic difference between strains of *X. vasicola* pv. *vasculorum* obtained from corn and strains obtained from sugarcane is the loss of the *xopAf* effector in strains obtained from corn (Lang et al. 2017).

Another xanthomonad, *Xanthomonas campestris*, has been recovered from asymptomatic sugarcane (Velázquez et al. 2008), clover (Burch and Sarathchandra 2006), and citrus (Araujo et al. 2002), although there have been some challenges identifying and taxonomically classifying *X. campestris* and *X. vasicola* pv. *vasculorum* (Bradbury, 1986; Qhobela and Claflin, 1992; Wasukira et al. 2014; Karamura et al. 2015; Lang et al. 2017), therefore the xanthomonad recovered from these hosts may not be *X. campestris*.

Several prairie grasses, weeds, turfgrasses, ornamental plants, and alternative crop species can be found growing in or near cornfields in the United States (Weaver and Bruner 1948, Stubbendieck et al 1994). Because this pathogen was only recently reported in the United States, the host range of *X. vasicola* pv. *vasculorum* on plants commonly found in the United States has not yet been investigated. Given the wide host range of this bacterium, there is a possibility that it may be capable of infecting other plants that grow in or near corn fields. If this proves to be true, it would make management more difficult by allowing the pathogen to survive in alternative hosts while corn is not being grown in the field.
In previous host range studies of *X. vasicola pv. vasicola*, only symptomatic plants were considered hosts. Additionally, only symptomatic plants found in field settings have been considered hosts of *X. vasicola pv. vasicola*. If *X. campestris*, a related xanthomonad, is capable of endophytically colonizing certain hosts without eliciting symptom development, it is possible that *X. vasicola pv. vasicola* is able to do this as well. This experiment was designed to test several plant species found in or near United States corn fields to determine their potential as both symptomatic and asymptomatic alternative hosts of *X. vasicola pv. vasicola*, and to determine the susceptibility of symptomatic hosts under field conditions.

**Materials and Methods**

**Greenhouse Conditions**

The experiments were conducted in the University of Nebraska-Lincoln Plant Pathology greenhouses. Average temperatures in the greenhouses were 28 °C during the day and 22 °C at night, and there was 16 hours of light and 8 hours of darkness. Average relative humidity in the greenhouse was 40%. Plants were watered daily, and pest control was performed as needed. To avoid dispersal of the pathogen via plant to plant contact, pots were spaced at least 12.7 cm apart, which prevented plant to plant contact.

**Strain preparation**

The strain of *X. vasicola pv. vasicola* was obtained from an infested field in Clay County, Nebraska during the 2016 growing season, and stored in -80°C storage vials containing a proprietary storage solution manufactured by Microbank (Pro-Lab Diagnostics Inc., Toronto, Canada). The strain was confirmed to be *X. vasicola pv.*
*vasculorum* via a PCR assay (Lang et al. 2017). Virulence was confirmed on corn hybrid DKC 61-88 at the fourth vegetative growth stage, with plants developing symptoms 5-7 days after spray inoculation with a ca. $10^8$ CFU/mL suspension of the strain (Korus et al. 2017).

In this study, the strain was removed from cold storage and streaked onto nutrient broth yeast extract (NBY) agar (Vidaver 1967) to obtain isolated colonies. After 48 hours of incubation at 27°C, a single colony of the strain was confluently streaked onto fresh nutrient broth yeast extract agar plates. After the plates were incubated at 27°C for 72 hours, the bacteria were scraped off of the plates with a sterile plastic scraper, and mixed into sterile water (250 ml of sterile water per plate). This mixture was used for the inoculations. Serial dilutions of the mixtures confirmed that they consistently contained ca. $10^8$ CFU/mL of *X. vasicola pv. vasculorum* in the sterile water.

**Experimental Design**

This experiment was repeated once in space. The experimental design was a completely randomized block design, where each species was a treatment, and treatments were blocked by inoculation date. Susceptible corn hybrid DKC 61-88 was included in each inoculation block as a positive control to ensure that the inoculum was virulent. Five experimental replicates, (Langemeier et al. 2014) of various crops, weeds, prairie grasses, and turf and ornamental grass species found throughout the United States were planted into steam pasteurized soil in clay pots 15.24 centimeters in diameter. Each pot was considered an experimental replicate. Planting depth varied by seed size (Newman and Moser 1987; Kirby 1993; Dawson and Bruns 1962; Redman and Qi 1992). After
emergence, plants were thinned to 5 plants per pot. When four leaves on at least 50% of plants of each species were fully extended, plants were spray inoculated with a ca. $10^8$ CFU/mL suspension of strain 201600401X in sterile water. Plants were sprayed until runoff, which deposited roughly 10 mL of inoculum per pot, then maintained in a humidity chamber at 100% humidity for twelve hours overnight after inoculation. Humidity was monitored with an AcuRite (Primex Family of Companies, Chaney Instrument Co., Lake Geneva, WI) temperature and humidity sensor. After 12 hours, plants were removed from the humidity chamber, and maintained in the greenhouse. At the time of inoculation, the top leaf on each plant was marked to note which leaves were present at the time of inoculation. Plants were monitored for symptoms every 48 hours for up to 21 days post inoculation. Negative controls of each species were inoculated with sterile water, and maintained under similar conditions in a separate greenhouse to prevent accidental inoculation with the pathogen (Langemeier et al. 2014).

*Examination of symptomatic plants*

When symptoms were observed on an unreported host, an excised piece of symptomatic leaf tissue approximately 3 cm by 3 cm was excised and examined for bacterial streaming at 200X magnification to verify that the symptoms were caused by a bacterium. If bacterial streaming was observed, symptom severity data was estimated 21 days post inoculation as the percent of the leaf area on each plant covered by lesions. Three symptomatic leaves were also collected per pot for pathogen isolation.

The pathogen was isolated from 9 cm$^2$ symptomatic tissue that was cut and surface disinfested with a 50:50 mixture of 70% ethanol and 10% hypochlorite (Schulz et
al. 1993) for 30 seconds. The tissue was then washed 3 times for 30 seconds with 8 mL of sterile water in 10 mL centrifuge tubes to ensure that sterilizing solution was removed (Lang et al. 2017). To prevent cross contamination between samples, centrifuge tubes were disinfested for 30 seconds with 70% ethanol, and rinsed 3 times with sterile water between samples. The efficacy of the surface sterilization solution was verified with the imprint method by Schulz et al. (1993). Disinfested leaf tissue was placed in 200 µl of sterile water in a 500 µl microcentrifuge tube, and cut five times with flame sterilized scissors to allow the bacteria to flow out of the tissue and into the water. One hour after leaf samples were cut in the sterile water, a sterile inoculation loop was placed in the sterile water and used to streak the bacterial solution onto an NBY plate to obtain isolated colonies. Plates were observed for bacterial growth every 24 hours for up to five days after isolation (Korus et al. 2017, Lang et al. 2017).

Suspect bacterial colonies were picked from the plate with sterile toothpicks and suspended in 100 µl of DNAse and protease free water that was previously filtered through a 0.2 micron filter and autoclaved (Fisher Bioreagents, Waltham, MA). The bacterial suspensions were used as templates in PCR assays developed by Lang et al. 2017, with primers 5′CAAGCAGAGCATGGCAAAC3’ and 5′CACGTAGAACCGGTCTTTGG3’ (Integrated DNA Technologies, Coralville, IA). 12.5 µl of commercially prepared DreamTaq 2X PCR Master Mix (Thermo Scientific Waltham, MA) was substituted for the reaction buffer, dNTP, and GoTaq DNA polymerase mixture. These PCR assays were used to confirm the colonies to be X. vasciola pv. vasculorum.
Isolation and quantification of *X. vasicola pv. vasculorum* in asymptomatic plants

Twenty-one days post inoculation, five leaves were collected per pot (Gagne-Bourgue et al. 2012), and it was noted if the leaf was present at the time of inoculation. Entire leaves were dried for 3 days at 27°C in a drying oven and weighed. Leaf weights were recorded to determine population densities per gram of leaf tissue. Leaves were surface sterilized in a 50:50 mixture of 70% ethanol and 10% hypochlorite for 30 seconds (Schulz et al. 1993), and washed 3 times for 30 seconds in sterile water (Lang et al. 2017). Leaves were placed in 300 µl of sterile water in 500 µl microcentrifuge tubes and cut with flame sterilized scissors while in the water to allow bacteria to flow out of the leaf tissue. To quantify the population densities of *X. vasicola pv. vasculorum*, after 1 hour the mixture of water and bacteria that flowed out of the leaf material was serially diluted until a 10^-8 concentration was reached. One hundred µl of each dilution was pipetted onto five replicate NBY plates, and spread with a sterile inoculation loop (Gagne-Bourgue et al. 2012). Plates were monitored every 24 hours for up to five days for suspect colonies. Suspect colonies were counted on the plates, and identified as *X. vasicola pv. vasculorum* with the same PCR procedure used to verify the presence of *X. vasicola pv. vasculorum* in symptomatic tissue. The number of bacterial colonies identified as *X. vasicola pv. vasculorum* was used to estimate the number of colony forming units present per gram of leaf tissue.

**Field study**

A field study was conducted to determine the likelihood of infection of susceptible hosts (identified in earlier greenhouse experiments) by *X. vasicola pv.*
vasculorum in a natural setting. The study was a completely randomized design repeated in two different locations, where each plant was an experimental unit. Experimental units were randomly assigned a position in the field with a number generator, where each number generated corresponded to a position in the field. Some hosts, including shattercane, johnsongrass, and yellow nutsedge, were not tested in this experiment, as there was potential for them to become invasive and challenging to control in the fields. Additionally, rice and sand bluestem were not included, as they had not yet been identified as alternative hosts when the field experiment was conducted.

After symptomatic alternative hosts were identified, fifteen replicates of each host species, including big bluestem, bristly foxtail, green foxtail, indiangrass, little bluestem, oat, orchardgrass, and timothy, were individually planted into 7.62 centimeter plastic pots containing pasteurized soil and grown in the University of Nebraska-Lincoln Plant Pathology greenhouse complex under the previously mentioned growing conditions until they reached the two-leaf stage. At this stage, plants were acclimated to ambient outdoor conditions by placement outside the greenhouse for 2 hr every morning for three days, then 5 hours from the morning into early afternoon daily for three days, then 8 hours per day daily for five days. After acclimation, fifteen replicates of each species were transplanted between corn rows in a no-till, continuous corn field known to be infested with *X. vasicola pv. vasculorum* at the University of Nebraska South Central Agricultural Laboratory, Clay Center, NE. The transplantation occurred on June 28, 2017, when the corn was at the ninth vegetative growth stage. To reduce stress on the transplants, the field was irrigated with overhead irrigation the same day as the transplant. Plants were
asymptomatic at the time of transplanting, and while the area was not inoculated, bacterial leaf streak symptoms were evident in the surrounding corn plants at approximately 30% severity.

Simultaneous to the field experiments, plants of each species were maintained in the greenhouse as negative controls to ensure that infection would not occur as a result of contamination in the greenhouse or during acclimation before transplanting. All plants were examined for symptoms at two week intervals until they began to senesce after approximately 12 weeks. Microscopic examination of suspect symptomatic leaf tissue, isolation attempts, and confirmation of the identity of the pathogen were conducted with the same methods as used in the greenhouse tests described above.

Statistics
ANOVA was conducted on all trials (α=0.05) in R version 3.4.0.

Results

Greenhouse Experiment

In the greenhouse experiment, 13 out of 56 tested plant species displayed typical symptoms of infection by *X. vasicola* pv. *vasculorum* (Tables 1-4). These symptoms included yellow to brown lesions with wavy or straight margins between the leaf veins. All positive control corn plants inoculated with the bacterium also displayed the typical symptoms, and disease was confirmed in all symptomatic corn plants via bacterial streaming observations and PCR, indicating that the inoculum was virulent at each inoculation and greenhouse conditions allowed symptom expression. Johnsongrass (*Sorghum halepense*), grain sorghum (*Sorghum bicolor* L. ‘TX430’), and shattercane
(Sorghum bicolor) plants displayed similar streaking at 100% infection incidence, red to purple in color, which is typical for these species. The X. vasicola pv. vasculorum specific PCR assay, along with observations of bacterial streaming from infected leaves confirmed that these streaks were caused by X. vasicola pv. vasculorum. Negative controls inoculated with water showed no symptoms, and the pathogen was not recovered from or detected in the leaves of plants inoculated with water.

Severity data for the two greenhouse trials were combined, as there was no interaction between the two trials in severity (p=0.7933). There was no difference in symptom severity between the inoculation blocks (p=0.606), however differences in symptom severity between species were significant (p<0.0001). On the previously untested species symptoms were least severe on yellow nutsedge at 4% severity, and most severe on johnsongrass at 27% severity. Disease severity on corn was significantly higher than disease severity on all other species at 28%. In both greenhouse trials, disease incidence was 100% for every symptomatic species except for sand bluestem, with an overall incidence of 50%, and timothy, with an overall incidence of 80% (Table 5).

Three asymptomatic hosts were identified including downy brome (Bromus tectorum), tall fescue (Festuca arundinacea ‘Cajun II’), and western wheatgrass (Pascopyrum smithii (Rydb.)). The pathogen was recovered from some inoculated plants, but was not recovered from or detected in any non-inoculated controls. Pathogen recovery was positive in 60% of western wheatgrass plants, 60% of downy brome plants, and 30% of tall fescue plants (data not shown). The pathogen was only confirmed in the lower leaves of the plants, which were present at the time of inoculation. There was no
interaction between the two repeats of the trial (p=0.937), or inoculation timing blocks (p=0.966). Population densities of *X. vasicola* pv. *vasculorum* detected varied by species (p<0.0001) with $\log_{10} 5.8$ CFU/gram of leaf tissue in downy brome and $\log_{10} 5.7$ CFU/g in tall fescue. The pathogen was present at an average population density of $\log_{10} 5.1$ CFU/g in western wheatgrass. The average $\log_{10}$ transformed concentration in symptomatic corn was 9.2 CFU/g (Figure 1).

*Field Experiment*

A low incidence of symptom development occurred in the field in two species, big bluestem and bristly foxtail. Big bluestem and bristly foxtail developed disease symptoms at 8 and 17% incidence, respectively. Average disease severity in big bluestem and bristly foxtail were 5 and 15%, respectively (Table 6). *X. vasicola* pv. *vasculorum* was not recovered from any asymptomatic plants transplanted in the field. Plants maintained as negative controls in the greenhouse did not develop symptoms, and the bacterium was not recovered from or detected in any of these plants.

*Discussion*

Sixteen of 56 (29%) tested plants that can be in or near corn fields were found to be symptomatic when inoculated with *X. vasicola* pv. *vasculorum* in a greenhouse setting. While this part of the experiment gives an indication of the bacterium’s virulence potential under optimum conditions for infection, these conditions will not likely happen in a natural environment. The results of the field study show that while symptoms may develop under some field conditions, the incidence and severity may be much lower than in the greenhouse environment, and the chance of any disease development is low, but
possible. During the 2017 growing season when the field experiment was conducted, overall incidence and severity of bacterial leaf streak in area corn was less than that observed during the 2016 and 2018 growing seasons (personal communication, T. Jackson-Ziems). A greater number of species may have become infected, or at a greater incidence and/or severity had field conditions been more conducive for disease development.

*X. vasicola pv. vasculorum* was virulent on several alternative hosts, however the symptoms were not as severe as symptoms observed on positive control corn plants. These results are consistent with research by Lang et al. (2017), which indicated that, when isolated from corn, the pathogen was more virulent on corn than on sorghum or sugarcane. Thus, while the strain of *X. vasicola pv. vasculorum* used in this study may be virulent on other hosts, it is possible that it’s better adapted to infect corn. Interestingly, johnsongrass in this study also displayed severe disease symptoms, although not as severe as symptoms on corn. Symptoms on johnsongrass were more severe than on grain sorghum or shattercane, despite these three plants being closely related. This demonstrates the need to examine the susceptibility of closely related plants, as well as cultivars and varieties of plant species to infection by *X. vasicola pv. vasculorum*. For example, the variety of tall fescue used in this experiment is more commonly found in pastures and prairies, and is not a variety that has been bred for use in home lawns. Examination of the potential for tall fescue varieties grown in home lawns to harbor the pathogen is needed in order to determine the potential of *X. vasicola pv. vasculorum* to survive in lawns.
The pathogen was detected within the leaves of asymptomatic hosts, but was present at low population densities compared to that in symptomatic corn. According to Vidaver and Lambrecht (2004), population densities of $10^6$ CFU are required for a plant associated bacterium to be pathogenic or to be a biological control agent. With an average population density of $10^6$ CFU/g in tall fescue, it is possible that *X. vasicola* pv. *vasculorum* could have a significant effect on plant health or endophytic microbe communities in tall fescue. With average population densities of $10^5$ CFU/g in downy brome and western wheatgrass, *X. vasicola* pv. *vasculorum* is less likely to impact plant health and microbiome composition. Population densities within the plants can vary due to the environmental factors as well, so these populations could possibly be present in higher or lower densities in a field setting, which could change the bacterium’s potential to affect the host. Additionally, Peros (1988) demonstrated that the aggressiveness of *X. vasicola* pv. *vasculorum* varies among strains, although all strains tested by Peros et al. displayed some level of virulence on all known hosts.

Pathogen recovery was only successful when isolating from leaves that were present at the time of inoculation. This suggests that, unlike in sugarcane, the pathogen does not move systemically within these plant species, although in the field it seems that symptoms move from the lower leaves to the upper canopy of the plant. This movement is likely due to the splashing of rain and irrigation in the field, which did not occur in the greenhouse. The apparent lack of systemic movement could be due to the difference in morphology and physiology between the alternative hosts and sugarcane (North et al.).
1935). Alternatively, it could be due to the loss of the XopAf effector and lack of TAL effectors in *X. vasicola* pv. *vasculorum* strains obtained from corn (Lang et al. 2017).

The results of this study have multiple implications for the management of this disease. Native grasses are commonly found in pastures, which can be baled and transported to other areas or states for use as livestock feed. If native grasses are symptomatically or asymptomatically infected with this bacterium, transportation of baled grasses could potentially be a source of spread. The pathogen may also be able to survive when corn is not being grown in a field by infecting grass weeds or host crops present in the field or in surrounding areas.

*X. vasicola* pv. *vasculorum* has an extensive host range, which includes several monocots commonly grown in the United States. The host range includes plants from both Poaceae and Cyperaceae, as well as palm and bamboo species (Ricaud and Autrey, 1969). Based on these findings, it is possible that other, untested plants may also be alternative hosts. Further research is needed to determine if the pathogen is capable of surviving within other monocotyledonous and dicotyledonous plants. Research into how pathogen concentrations change over time in asymptomatic hosts would also give an indication of the possibility for these hosts to harbor the pathogen long-term, and contribute to its spread. Additionally, research into pathogen survival over time, specifically in perennial grass hosts such as big bluestem and little bluestem, is necessary to determine if the longer survival of these plants allows for longer survival of the pathogen.
Multiple rice cultivars were examined in this study due to the potential significance *X. vasicola* pv. *vasculorum* as a pathogen in rice growing regions in Arkansas. With the exception of rice, only single cultivars of each species were tested. Other cultivars may be more or less susceptible, and should be evaluated against *X. vasicola* pv. *vasculorum*. No plants of these species were sampled or surveyed in the field, but should be considered to better understand whether infection is occurring in pastures, ditches or conservation reserve program (CRP) acres near corn fields. Notably, *X. vasicola* pv. *vasculorum* has not been found infecting grain sorghum in Nebraska or Kansas, including in grain sorghum fields adjacent to corn fields with bacterial leaf streak (Personal communication, Tamra Jackson-Ziems, University of Nebraska-Lincoln and Doug Jardine, Kansas State University), indicating that *X. vasicola* pv. *vasculorum* may not always cause symptom development in grain sorghum.

In summary, this research has shown that eleven monocotyledonous species displayed symptoms when infected with *X. vasicola* pv. *vasculorum*, and three remained asymptomatic when infected by *X. vasicola* pv. *vasculorum*. Further research is needed to determine if other, untested species can also harbor the pathogen and their implications for disease development and overwintering.

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grass seed; Dr. Amit Jhala and Kasey Schroeder for weed seed; and Michayla Goedeken and Jen Foster for technical assistance.
Literature Cited


Table 1: Crops tested for susceptibility to infection by *Xanthomonas vasicola* pv. *vasculorum*, including host and non-host plants.

<table>
<thead>
<tr>
<th>Symptomatic hosts</th>
<th>Asymptomatic hosts</th>
<th>Non-hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Grain Sorghum <em>(Sorghum bicolor</em> L. ‘TX430’)</td>
<td>No tested species</td>
<td>Barley, winter <em>Hordeum vulgare</em> L. ‘NB14430’</td>
</tr>
<tr>
<td>Oat <em>Avena sativa</em> L. ‘Jerry’</td>
<td></td>
<td>Cereal Rye <em>Secale cereal</em> L. ‘Elbon’</td>
</tr>
<tr>
<td>Rice <em>Oryza sativa</em></td>
<td></td>
<td>Foxtail Millet <em>Setaria italica</em></td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Switchgrass</em> <em>Panicum virgatum</em> L.</td>
</tr>
<tr>
<td></td>
<td></td>
<td><em>Triticale</em> <em>Triticosecale</em> Wittm. ex A. Camus ‘NT13416’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Wheat, winter <em>Triticum aestivum</em> L. ‘Settler CL’</td>
</tr>
</tbody>
</table>
**Table 2**: Weeds tested for susceptibility to infection by *Xanthomonas vasicola pv. vasculorum*, including host and non-host plants.

<table>
<thead>
<tr>
<th>Symptomatic hosts</th>
<th>Asymptomatic hosts</th>
<th>Non-hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Green Foxtail</td>
<td>Setaria viridis (L.) P. Beauv.</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Johnsongrass</td>
<td>(Sorghum halepense)</td>
<td>Giant Foxtail Setaria faberi Herrm.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Shattercane</td>
<td>(Sorghum bicolor L.)</td>
<td>Large Crabgrass Digitaria sanguinalis (L.) Scop.</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yellow Nutsedge</td>
<td>Cyperus esculentus L.</td>
<td>Palmer Amaranth Amaranthus palmeri</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sandbur Cenchrus longispinus (Hack.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Smooth Brome Bromus inermis Leyss. ‘VNS’</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Yellow Foxtail Setaria pumila (Poir.) Boem. &amp; Schult.</td>
</tr>
</tbody>
</table>
Table 3: Prairie grasses tested for susceptibility to infection by *Xanthomonas vasicola pv. vasculorum*, including host and non-host plants.

<table>
<thead>
<tr>
<th>Symptomatic hosts</th>
<th>Asymptomatic hosts</th>
<th>Non-hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>Big Bluestem <em>Andropogon gerardii</em> Vitman ‘Champ’</td>
<td>Western Wheatgrass <em>Pascopyrum smithii</em> (Rydb.)</td>
<td>Annual Ryegrass <em>Lolium multiflorum</em> L. ‘Gulf’</td>
</tr>
<tr>
<td>Indiangrass <em>Sorghastrum nutans</em> (L.) Nash ‘Holt’</td>
<td></td>
<td>Bluegrama <em>Bouteloua gracilis</em> (Wild. ex Kunth.) Lag. ex Griffiths ‘Bad River’</td>
</tr>
<tr>
<td>Little Bluestem <em>Schizachyrium scoparium</em> (Michx.) Nash ‘Blaze’</td>
<td></td>
<td>Creeping Bentgrass <em>Agrostis stolonifera</em> L.</td>
</tr>
<tr>
<td>Orchardgrass <em>Dactylis glomerata</em> L. ‘Latar’</td>
<td></td>
<td>Creeping Foxtail <em>Alopecurus arundinaceus</em> Poir. ‘Garrison’</td>
</tr>
<tr>
<td>Sand Bluestem <em>Andropogon hallii</em> (Hack.)</td>
<td></td>
<td>Crested Wheatgrass <em>Agropyron cristatum</em> L. Gaertn.</td>
</tr>
<tr>
<td>Timothy <em>Phleum pratense</em> L. ‘Climax’</td>
<td></td>
<td>Festulolium <em>Festulolium loliaceum</em> (Huds.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Green Needle <em>Nassella viridula</em> (Trin.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Junegrass <em>Koeleria macrantha</em> (Ledeb.)</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Meadow Brome <em>Bromus riparius</em> Rehmann ‘Fleet’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Prairie Sandreed <em>Calamovilfa longifolia</em> (Hook.) Scribn. ‘Goshen’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Pubescent Wheatgrass <em>Thinopyrum intermedium</em> [Host] Barkworth &amp; D.R. Dewey ‘Manska’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Reed Canary <em>Phalaris arundinacea</em> L.</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sand Dropseed <em>Sporobolus cryptandrus</em> (Torr.) A. Gray ‘VNS’</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Sideoats Grama <em>Bouteloua curtipendula</em></td>
</tr>
<tr>
<td></td>
<td>(Michx.) Torr. ‘Butte’</td>
<td></td>
</tr>
<tr>
<td>------------------</td>
<td>------------------------</td>
<td></td>
</tr>
<tr>
<td>Slender Wheatgrass</td>
<td><em>Elymus trachycaulus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Link.)</td>
<td></td>
</tr>
<tr>
<td>Tall Wheatgrass</td>
<td><em>Thinopyrum ponticum</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Podp.)</td>
<td></td>
</tr>
<tr>
<td>Thickspike Wheatgrass</td>
<td><em>Elymus lanceolatus</em></td>
<td></td>
</tr>
<tr>
<td></td>
<td>(Scribn. &amp; J.G. Sm.)</td>
<td></td>
</tr>
<tr>
<td>Virginia Wildrye</td>
<td><em>Elymus virginicus</em> L.</td>
<td></td>
</tr>
</tbody>
</table>
**Table 4:** Turf and ornamental grasses tested for susceptibility to infection by *Xanthomonas vasicola* pv. *vasculorum*, including host and non-host plants.

<table>
<thead>
<tr>
<th>Symptomatic hosts</th>
<th>Asymptomatic hosts</th>
<th>Non-hosts</th>
</tr>
</thead>
<tbody>
<tr>
<td>No tested species</td>
<td>Tall Fescue <em>Schedonorus arundinaceus</em> (Schreb.) Dumor., nom. cons. ‘Cajun’</td>
<td>Bermudagrass <em>Cynodon dactylon</em> (L.) Pers.</td>
</tr>
<tr>
<td></td>
<td>Buffalograss <em>Buchloe dactyloides</em> (Nutt.) Engelm.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Daylily <em>Hemerocallis</em> L.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Kentucky Bluegrass <em>Poa pratensis</em> L.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Ornamental Pearl Millet <em>Pennisetum glaucum</em> (L.) R. Br.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Perennial Ryegrass <em>Lolium perenne</em> L.</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Zoysiagrass <em>Zoysia matrella</em></td>
<td></td>
</tr>
</tbody>
</table>
Table 5: Symptom incidence severity in the greenhouse study after spray inoculation with a c.a. $10^8$ CFU/mL suspension of *Xanthomonas vasicola* pv. *vaccinorum* in water. Incidence and severity were combined over two trials. Severity was determined via visual estimation, and incidence was calculated as the percent of plants displaying symptoms. Corn was included as a positive control. Letters indicate significant differences within a column.

<table>
<thead>
<tr>
<th>Species</th>
<th>Average Severity (% leaf area covered by lesions)</th>
<th>Incidence (% plants infected)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Big Bluestem</td>
<td>15 de</td>
<td>100</td>
</tr>
<tr>
<td>Bristly Foxtail</td>
<td>7 b</td>
<td>100</td>
</tr>
<tr>
<td>Corn</td>
<td>27 f</td>
<td>100</td>
</tr>
<tr>
<td>Grain Sorghum</td>
<td>7 bc</td>
<td>100</td>
</tr>
<tr>
<td>Green Foxtail</td>
<td>7 bc</td>
<td>100</td>
</tr>
<tr>
<td>Indiangrass</td>
<td>13 cd</td>
<td>100</td>
</tr>
<tr>
<td>Little Bluestem</td>
<td>12 d</td>
<td>100</td>
</tr>
<tr>
<td>Oat</td>
<td>18 de</td>
<td>100</td>
</tr>
<tr>
<td>Orchardgrass</td>
<td>6 ab</td>
<td>100</td>
</tr>
<tr>
<td>Rice</td>
<td>8 b</td>
<td>100</td>
</tr>
<tr>
<td>Sand Bluestem</td>
<td>12 ab</td>
<td>50</td>
</tr>
<tr>
<td>Timothy</td>
<td>4 a</td>
<td>80</td>
</tr>
<tr>
<td>Shattercane</td>
<td>4 ab</td>
<td>100</td>
</tr>
<tr>
<td>Johnsongrass</td>
<td>18 e</td>
<td>100</td>
</tr>
<tr>
<td>Yellow Nutsedge</td>
<td>4 a</td>
<td>100</td>
</tr>
</tbody>
</table>
Figure 1: Recovered quantities of *X. vasicola* pv. *vasculorum* per gram of dry leaf tissue after inoculation with a ca. $10^8$ CFU/mL suspension of *Xanthomonas vasicola* pv. *vasculorum* in water. Symptomatic corn was used as a positive control to ensure inoculum was viable.
Table 6: Incidence and severity of bacterial leaf streak on susceptible hosts in a field setting. Plants were not inoculated, but grew in corn residue infested with *X. vasicola* pv. *vasculorum* and near corn plants infected with *X. vasicola* pv. *vasculorum*. Incidence was calculated as the percent of plants displaying symptoms, and severity was a visual estimate of leaf area covered by lesions. Incidence and severity were averaged over two trials. Letters indicate significant differences within a column.

<table>
<thead>
<tr>
<th>Species</th>
<th>Average Severity (%)</th>
<th>Incidence (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Oat</td>
<td>0 a</td>
<td>0</td>
</tr>
<tr>
<td>Green Foxtail</td>
<td>0 a</td>
<td>0</td>
</tr>
<tr>
<td>Bristly Foxtail</td>
<td>5 b</td>
<td>17</td>
</tr>
<tr>
<td>Indiangrass</td>
<td>0 a</td>
<td>0</td>
</tr>
<tr>
<td>Orchardgrass</td>
<td>0 a</td>
<td>0</td>
</tr>
<tr>
<td>Big Bluestem</td>
<td>15 b</td>
<td>8</td>
</tr>
<tr>
<td>Little Bluestem</td>
<td>0 a</td>
<td>0</td>
</tr>
<tr>
<td>Timothy</td>
<td>0 a</td>
<td>0</td>
</tr>
</tbody>
</table>
CHAPTER III

DISTRIBUTION OF XANTHOMONAS VASICOLA PV. VASCULORUM IN NEBRASKA AND INVESTIGATION OF FACTORS ASSOCIATED WITH ITS DEVELOPMENT
Abstract

In 2016, the bacterium *Xanthomonas vasicola* pv. *vasculorum*, causing bacterial leaf streak, was reported on Nebraska corn, the first report in the United States. Prior to this report, the pathogen had only been reported on corn in South Africa. Since the initial report on Nebraska corn, *X. vasicola* pv. *vasculorum* has been reported in Argentina and Brazil. Because this pathogen had not been previously identified in the United States, a survey was initiated during the 2016-2018 growing seasons, primarily in Nebraska, to determine the distribution of the pathogen. The survey also examined factors that may be associated with disease development. Over 2,000 surveys were distributed to agribusiness professionals. Respondents were asked to submit a symptomatic leaf sample for confirmation of the pathogen, along with a completed survey on agronomic practices. Three hundred twenty-five surveys were returned with samples. Bacteria were isolated from samples, and PCR assays indicated that the pathogen was present in samples from 74 Nebraska counties. Analyses of survey responses with Random Forest indicated that irrigation, planting date, and crop rotation were the three most important predictors of a sample testing positive for the presence of *X. vasicola* pv. *vasculorum*. According to the classification and regression tree (CART) analysis, irrigation use, multiple years of corn in a crop rotation or a corn-sorghum rotation, plants being at the V7-R2 stage, and a planting date after May 1st were the most important predictors of a sample testing positive for the presence of *X. vasicola* pv. *vasculorum*.  


Introduction

*Xanthomonas vasicola* pv. *vasculorum* was first reported in 2016 causing bacterial leaf streak on Nebraska corn (*Zea mays*). It has since been reported in 9 other states, including Iowa and Illinois, meaning that the top three corn producing states by bushel have been affected by *X. vasicola* pv. *vasculorum* (Korus et al. 2017; USDA, NASS Crop Production Summary, 2016). In the United States, the pathogen has been reported in Colorado, Iowa, Illinois, Kansas, Minnesota, Oklahoma, South Dakota, Texas, and Wisconsin (Korus et al. 2017; Bowman et al. 2017; Smith et al. 2018). *X. vasicola* pv. *vasculorum* has also been reported causing bacterial leaf streak on corn in Argentina (Plazas et al. 2018) and Brazil (Augusto et al. 2018). The only previous report of this pathogen causing disease on corn was in South Africa (Dyer, 1949). The bacterium also causes gumming disease of sugarcane, a leaf blight and systemic vascular wilt (North, 1935). The pathogen has been reported in sugarcane growing areas around the world, but has not been confirmed on sugarcane in the United States. Although there has been some research into the epidemiology of this pathogen (North, 1935; Thompson 1957; Ricaud and Autrey, 1989; Wasukira et al. 2014; Karamura et al. 2015; Korus et al. 2017; Lang et al. 2017), agronomic practices that favor the survival of the pathogen and subsequent disease development in corn under United States environments are currently unknown.

Agronomic factors that may contribute to disease development can be explored with the Classification and Regression Tree, or CART (Breiman et al. 1984), and Random Forest (Breiman, 2001) analyses (Kim et al., 2002; Paul and Munkvold, 2004,
Langemeier et al. 2017). These analyses can be used to build decision trees, which can then be used determine which factors are the most important predictors of a sample from a field testing positive for a pathogen. Decision trees are made of nodes and branches, and can be based on survey data (Langemeier et al. 2017) or field observations over multiple growing seasons and locations (Paul and Munkvold, 2004). Within a decision tree, a node can be split into two more nodes, which makes the split node into a parent node and the nodes at each split into child nodes. Each node in the tree represents a factor addressed in the survey, and terminal nodes represent the positive or negative result in the test for the pathogen (Lewis 2000).

In the CART analysis, factors associated with nodes placed at the top of the tree have the largest segregation between survey responses. At these nodes, a high percentage of responses corresponding with samples from fields that tested positive for the pathogen were weighted towards one factor. This contrast decreases as nodes are placed near the bottom of the tree. Therefore, factors placed at the top of the tree are considered the most important predictors for disease development, with decreasing importance as the nodes are placed closer to the bottom of the tree, with the lowest nodes being the least important predictors (Langemeier et al. 2017). The advantage of CART is that it generates a single, easy to understand figure based on all of the data, however it is somewhat unstable, and can be error prone in some cases, as it has a tendency to over-fit the tree to a specific data set (Venables and Ripley 1997). Selecting a tree with a minimal cross validated error can prevent overfitting (Kohavi, 1995). Parameters such as minimum split and cost complexity factor also control tree growth and can be used to mitigate some of this issue.
Minimum split specifies a minimum number of observations at a node that must be present before attempting a split. The cost complexity factor specifies that the split must decrease the overall lack of fit by a certain factor before it can be attempted. These parameters ensure that the most informative nodes are kept in the tree, and the least informative nodes are pruned from the tree.

Random Forest is similar to CART, in that they both use decision trees generated by survey data to determine which factors are the most important predictors of disease development. However, Random Forest uses a random sampling of data points with replacement to generate many possible decision trees. It then uses an average of these trees to generate gini coefficients. The gini coefficients used in the Random Forest analysis relate to nodes on the trees that are generated. Gini coefficients represent the decrease in node impurity. Therefore, the higher the gini coefficient, the more pure the node is (Breiman 2001). Higher node purity indicates that survey responses corresponding with a field that tested positive for the pathogen leaned significantly towards one result, meaning that factor could be an important predictor for disease development. Factors based on survey results are then ranked based on gini coefficients, the factors with the highest gini coefficients being the most important predictors of disease development (Langemeier et al. 2017). One advantage of Random Forest is its ability to handle missing data, which can be done by imputing the data (Ishioka, 2013). Another advantage is that the random sampling of data points and average of many trees avoids overfitting to the specific dataset (Breiman, 2001). Given that CART generates one tree based on the entire dataset, and Random Forest averages many trees based on
subsamples of the dataset, it is advantageous to use both and take all results into account when analyzing a dataset (Langemeier et al. 2017).

*X. vasicola* pv. *vasculorum* had not been previously reported in the United States, therefore its distribution was unknown. Not knowing the extent of the pathogen’s distribution created obstacles when attempting to effectively educate agribusiness professionals on this pathogen. The lack of data on agronomic practices that may be contributing to or exacerbating disease development was another problem, as data were needed to focus future research directions. To address these issues, a survey was conducted over the 2016-2018 corn growing seasons. The objectives of the survey were to determine the distribution of the pathogen, and to use data obtained from the surveys to gain a better understanding of which agronomic and environmental factors may be associated with disease development.

**Materials and Methods**

*Survey distribution and generation of questions*

A survey was developed to gain a better understanding of which agronomic practices and environmental factors were commonly occurring in infested fields. Survey questions were generated based on field observations by agricultural scientists and information about other xanthomonads similar to a previous survey by Langemeier et al. (2017). This survey included questions about crop rotation and cover crops (O’Garro et al. 1997), tillage and residue cover (Gent et al. 2005), soil type (Schaad et al. 1974) irrigation (Schwartz et al. 2003; Gent et al. 2005), proximity to other plants such as weeds and Conservation Reserve Program acres (Gent et al. 2005), crop growth stage and
symptom distribution, environmental stressors, and agricultural chemical use (Langemeier et al. 2017). Surveys were distributed to farmers, Nebraska Extension personnel, crop consultants, and other agribusiness professionals. Distribution of surveys took place largely at corn disease update meetings, crop scout trainings, and other informational events hosted by University of Nebraska-Lincoln Extension. Respondents were asked to submit a sample for pathogen isolation and confirmation, along with the completed survey. The focus of the survey was corn fields in Nebraska, however some respondents submitted samples from other states. The method of survey distribution created a self-selected sample for the survey, as potential respondents were most likely to attend meetings where surveys were distributed if they were aware of a potential issue in their fields. However, this was an optimal method for determining the distribution of the pathogen and obtaining a geographically diverse collection of strains of *X. vasicola* pv. *vasculorum*.

**Testing for Pathogen Presence**

Bacterial pathogen presence was confirmed by microscopic observation of bacterial streaming from suspect lesions. A 1 by 1 centimeter portion was excised out of the suspect lesion with a flame-sterilized razor blade and placed in sterile water on a microscope slide. A cover slip was placed over the excised leaf portion and observed at 200X with a compound microscope. If bacterial streaming was observed, a 3 by 3 centimeter portion of symptomatic leaf tissue was excised with flame-sterilized scissors and placed in a 50:50 mixture of 10% hypochlorite and 70% ethanol for 30 seconds (Schultz et al. 1993). The tissue was rinsed three times in sterile water for 30 seconds
each time before being placed in a 500 µL microcentrifuge tube with 200 µL of sterile water. In the sterile water, the leaf portion was cut five times with flame sterilized scissors to allow the bacteria to stream into the water. One hour after cutting, a sterile loop was used to streak the suspension onto a nutrient broth yeast extract (Vidaver, 1967) agar plate (Lang et al. 2017). Plates were monitored for up to five days for suspect colony growth (Korus et al. 2017). Yellow, mucoid colonies, the typical morphology of *X. vasicola* pv. *vasculorum*, were picked from the plate with sterile toothpicks and placed in 100 µL of DNase and protease free water previously filtered through a 0.2 micron filter and autoclaved (Fisher Bioreagents, Waltham, MA). These suspended bacterial colonies were used as the templates in the *X. vasicola* pv. *vasculorum* specific PCR assay developed by Lang et al. (2017), with primers 5’CAAGCAGAGCATGGCAAC3’ and 5’CACGTAAGACCGGGTTTG3’ (Integrated DNA Technologies, Coralville, IA).

12.5 µl of pre-mixed DreamTaq 2X PCR Master Mix (Thermo Scientific Waltham, MA) was used in place of the master mix used by Lang et al. (2017).

Statistics

The CART and Random Forest packages in R version 3.4.0 were used to generate a classification tree and to rank factors based on their gini coefficients, respectively. Chi-squared tests of independence were conducted in the same version of R. Factors were named based on survey questions. For example, the question “What is the cropping rotation in this field?” would correspond to the factor “crop rotation”. Only questions answered by greater than 50% respondents were examined with CART and Random Forest (Langemeier et al. 2017). Questions answered by less than 50% of respondents
were examined with a Chi Square analysis in R version 3.4.0. Questions such as estimated residue cover, distribution of symptoms, incidence, and environmental stressors were subjective, and relied on the judgement of each individual submitter. No statistical analyses were conducted on these questions due to their subjective nature, however figures were generated based on these questions. These figures were generated in R version 3.4.0.

Questions on planting population and planting date were open ended, and therefore needed to be grouped for analysis. These groups were created based on the groupings in the Goss’s Wilt survey by Langemeier et al. (2017). Seeding rates ranged from 32,123 to 93,900 plants/ha, and were grouped into 10 groups. Group 1 had seeding rates of less than 54,999 plants/ha, group 2 had seeding rates of 55,000 to 59,999 plants/ha, group 3 had seeding rates of 60,000 to 64,999 plants/ha, group 4 had seeding rates of 65,000 to 69,999 plants/ha, group 5 had seeding rates of 70,000 to 74,999 plants/ha, group 6 had seeding rates of 75,000 to 79,999 plants/ha, group 7 had seeding rates of 80,000 to 84,999 plants/ha, group 8 had seeding rates of 85,000 to 89,999 plants/ha, group 9 had seeding rates of 90,000 to 94,999 plants/ha, and group 10 had seeding rates of 95,000 to 100,000 plants/ha. Planting dates ranged from April 4th and June 5th, and were grouped into 5 groups for analysis. Group 1 was planted before April 21st, group 2 was planted between April 22nd and May 1st, group 3 was planted between May 2nd and May 12th, group 4 was planted between May 13th and May 23rd, and group 5 was planted after May 24th (Langemeier et al. 2017).
Fourteen variables were analyzed with a CART tree (Langemeier et al. 2017). The Random Forest analysis examined the same fourteen variables. In the Random Forest analysis, missing data was imputed with the na.roughfix function (Ishioka, 2013) in the Random Forest package. The data were fit with the Random Forest package, with each examined survey factor being the x-values and the result of the test for the presence of *X. vasicola* pv. *vasculorum* being the y-value. One hundred classification trees were averaged to determine the final output of the analysis.

For variables with a response rate of less than 50%, a contingency table was created, and a chi-square test was used to determine if the variable was independent of the sample test result at an alpha level of 0.05. Only variables including at least 10 responses from each mutually exclusive outcome in the contingency table were examined with the chi-square analysis (Langemeier et al. 2017). Questions that could not be analyzed with CART, Random Forest, or the chi-square analyses due to low response rates were not examined.

**Results**

Throughout the 2016-2017 corn growing seasons, 325 surveys, 16.25% of surveys distributed, were returned at least partially completed. Each survey also included a leaf sample. Of the samples received, 80% tested positive and 20% tested negative for the presence of *X. vasicola* pv. *vasculorum*. Positive samples received from the 2016-2018 growing seasons represented 74 counties in Nebraska (Figure 1), spanning from the Panhandle east to the counties bordering the Missouri River. Ninety-four percent of samples were from dent corn, 5% were from popcorn, and 1% were from sweet corn.
Seventy-four percent of respondents who submitted a sample testing positive for *X. vasicola* pv. *vasculorum* positive sample visually estimated the area in the sampled field that was covered with corn residue on a scale of 1-100 (n=192). Of the respondents, 31% rated the residue cover in their sampled field at 1-15; 30% rated their residue cover at 15-30; 15% of respondents had an estimated residue cover of 30-45; 14% had an estimated residue cover of 25-60; 6% of respondents estimated their residue cover at 60-75; and 3% of respondents rated their residue cover at upwards of 75 (Figure 2).

Seventy-seven percent of respondents estimated disease incidence as the percent of symptomatic plants in the field at the time of sample collection (n=200). Because this variable is subjective, and relies on the judgement of the individual respondent, it was not statistically analyzed. Twenty percent of respondents who submitted positive samples reported an incidence of 1-10%; 18% of respondents reported an incidence of 10-20%; 14% of respondents reported an incidence of 20-40%; 15% of respondents reported an incidence of 40-60%; 19% of respondents reported an incidence of 60-80%; and 14% of respondents reported an incidence of higher than 80% (*data not shown*).

Five of the 14 variables examined by the CART analysis were considered informative enough to be included in the tree. The CART analysis indicated that center pivot, lateral, gravity, and sub surface irrigation were most strongly associated with a sample testing positive for *X. vasicola* pv. *vasculorum*. Eighty-six percent of samples from fields that were irrigated tested positive for *X. vasicola* pv. *vasculorum*, and 14% tested negative. In non-irrigated fields, 42% of samples tested positive for *X. vasicola* pv. *vasculorum*, while 58% tested negative. Growth stage was also an important predictor.
When samples were from irrigated fields, 90% from plants in the V7-R3 growth stages tested positive for \textit{X. vasicola pv. vasculorum}, and 10% testing negative. When plants in irrigated fields were not in this range of growth stages, 71% of samples tested positive for \textit{X. vasicola pv. vasculorum}, while 29% tested negative. Crop rotation was an important factor in non-irrigated fields, with 88% of samples from non-irrigated fields with a continuous corn, corn-corn-soybean, or corn-grain sorghum fields testing positive for \textit{X. vasicola pv. vasculorum}, and 12% testing negative. Thirty-three percent of samples from non-irrigated fields with a corn-soybean, corn-soybean-wheat-soybean, or wheat-corn-fallow rotation tested positive for \textit{X. vasicola pv. vasculorum}, while 67% of samples from these fields tested negative. Eighty-eight percent of samples from irrigated fields with plants in the VE-V6 or R4 and later growth stages and planted after May 2nd tested positive for \textit{X. vasicola pv. vasculorum}, and 12% of samples from these fields tested negative. If fields under the same conditions were planted before May 2nd, 50% of samples tested positive, and 50% tested negative. In non-irrigated fields with a corn-soybean, corn-soybean-wheat-soybean, or wheat-corn-fallow rotation, tillage was an important predictor of disease. In these fields, if growers used minimum, no-till, or strip till 24% of samples tested positive, while 76% tested negative. If growers used conventional tillage in these same fields, 66% of samples tested positive and 33% of samples tested negative (Figure 3; Table 1).

According to the Random Forest analysis, irrigation type, planting date, crop rotation, tillage type, and growth stage were the strongest predictors of samples testing positive for \textit{X. vasicola pv. vasculorum} during the 2016-2017 corn growing seasons in
Nebraska (Figure 4). These variables were determined to be the most important predictors of a positive test result according to the gini coefficients generated by Random Forest (Figure 4). Five variables that were informative enough to be included in the CART tree were irrigation type, planting date, crop rotation, growth stage, and tillage type (Figure 3; Table 1). These five variables were also the five most important predictors of a sample testing positive for X. vasicola pv. vasculorum according to the Random Forest analysis (Figure 4; Table 1).

The chi-square analysis indicated that nitrogen application at planting may be correlated with a positive test for X. vasicola pv. vasculorum (p=0.001586). Other nutrient applications examined in the chi-square analysis included pre-plant (p=0.10) and at-plant (p=0.1153) potassium and pre-plant phosphorus (p=0.8747) applications, none of which affected samples testing positive for X. vasicola pv. vasculorum (Table 2). Herbicide applications were also analyzed for their potential impacts on samples testing positive with the chi-squared analysis. Application of a herbicide containing the active ingredient glyphosate was related to a positive test for X. vasicola pv. vasculorum (p=0.01725). No other herbicide active ingredients impacted samples testing positive, including acetochlor (p=0.5458), S-Metolachlor (p=0.2309), and atrazine (p=0.9372) (Figure 8). Application of a fungicide did not impact a positive test results (p=0.6649) at an alpha level of 0.05 (Table 3). There were not enough survey responses to examine the effects of different fungicide active ingredients. According to the chi-square analysis, neither a silt (p=0.8267) nor a clay soil type (p=0.06669) were significantly correlated with a positive test result at an alpha level of 0.05 (Table 4).
Insufficient responses were received for some questions to be examined by any analyses. These questions corresponded with variables that included weed density, inches of irrigation water applied up until the time of sampling, and micronutrient application. Other variables received a high response rate, however there were not enough responses in one or more of the outcomes in the contingency table to be used in the chi-square analysis. These variables included sand and loam-textured soils; pre-plant, in-season fertigate, in-season sidedress, and dry spread broadcast nitrogen application; in-season fertigate, in-season sidedress, dry spread broadcast potassium application; and at-plant, in-season fertigate, in-season sidedress, and dry spread broadcast phosphorus application. Weed species reported as problematic by less than 50% of growers were not included in the CART or random forest analyses. Questions on symptom distribution and environmental stressors that may have affected the plants were not examined due to their subjective nature.

Discussion

Results of both CART and random forest analyses indicated that irrigation, crop rotation, growth stage, tillage, and planting date were the most important predictors of disease development, although not in the same order (Figures 5, 6; Table 1). This is likely due to the inherent differences in the CART and random forest analyses. This result is consistent with the Goss’s bacterial wilt and blight survey conducted by Langemeier et al. (2017), which also showed that CART and random forest produced the same list of variables that were the most important predictors of disease, although they were not in the same order.
Samples were voluntarily submitted from fields and not randomly selected. Growers, consultants, and agribusiness professionals were asked to submit samples that they believed to be displaying bacterial leaf streak symptoms, therefore the proportion of positive samples reported in this survey is not necessarily a reflection of the proportion of Nebraska fields infested with *X. vasicola* pv. *vasculorum*. Additionally, some agronomic practices are better represented than others because of the greater emphasis and sample collection in some areas than others. For example, fields with higher yield potential, such as irrigated fields, were more likely to be scouted and samples collected than others. These results are only a reflection of what occurred in Nebraska fields during the 2016-2018 growing seasons, and other factors may influence disease development in different areas and during other growing seasons. However, results of this survey have shown that *X. vasicola* pv. *vasculorum* is widely distributed throughout Nebraska, despite the differences in environment and agronomic practices throughout the state.

Some results reported in this survey, such as percent residue cover and symptom distribution, are subjective as they rely on the respondent’s memory and their judgement. Such estimations and judgements can vary between individual respondents, therefore these variables were reported only as proportions, and no analyses were completed to determine their association with disease development. Residue ratings tended towards the lower end of the scale, with 31% of respondents reporting a residue rating of 1-15, a result that seems to contradict the idea that this pathogen is likely residue-borne (North et al. 1935) and overwintering populations may provide the initial inoculum for future
infections. Fifty-eight percent of respondents also reported a fieldwide distribution of symptoms (data not shown). Further field research in a more controlled experimental setting could produce a much more reliable dataset.

According to the random forest analysis, the most important predictor of a positive \textit{X. vasicola} pv. \textit{vasculorum} test result was irrigation (Figure 6). The CART analysis also determined that irrigation was strongly associated with a positive test result, however no specific type of irrigation was singled out as the most important predictor. The difference in association with disease was between irrigated versus non-irrigated fields (Figure 5). An increase in humidity can occur when extra moisture is introduced into a field via any type of irrigation (Boucher et al. 2004), and according to Jackson-Ziems et al. (2017), bacterial leaf streak disease severity is positively correlated with humidity levels. Increased humidity also causes plants to open their stomata (Lange et al. 1971). Korus et al. (2017) demonstrated that \textit{X. vasicola} pv. \textit{vasculorum} is capable of entering plants through natural openings, without wounding. Thus, increased humidity due to irrigation may favor infection by causing the stomata, which are natural openings on the plants, to remain open for longer periods of time. It has also been shown that Xanthomonas leaf blight of onion, caused by \textit{Xanthomonas axonopodis} pv. \textit{alii}, can survive and be spread in irrigation water (Gent et al. 2005). It is currently unknown if \textit{X. vasicola} pv. \textit{vasculorum} is also capable of surviving in irrigation water, however both CART and random forest indicate that there may be a connection.

Growth stage was an important predictor of \textit{X. vasicola} pv. \textit{vasculorum} presence in irrigated fields (Figure 5). This may be due to irrigated fields in Nebraska being more
likely to be scouted mid-season than non-irrigated fields (Tamra Jackson-Ziems, Personal Communication). This would explain why 197 *X. vasicola* pv. *vasculorum* positive samples from irrigated fields were received when plants were at the V10-R3 growth stage. Alternatively, initial infection may have occurred early in the season, and the V10-R3 growth stage could be when symptoms became severe enough to be noticed by growers in the 2016-2017 growing seasons. This is possible considering that Korus et al. (2017) demonstrated that plants could be infected as early as V4, and Jackson-Ziems et al. (2017) demonstrated that it was possible for symptoms to develop after inoculation at VE, albeit in a greenhouse setting.

Planting date was also determined to be an important predictor of a positive test in irrigated fields where plants were at VE-V6 or R4 and later stages. According to CART, a planting date on or after May 3rd was an important predictor of a sample testing positive in these fields (Figure 3). Paul and Munkvold (2004) demonstrated with a similar CART model that gray leaf spot development was influenced by a later planting date. The influence of the planting date could be connected with the growth stage of the plants and the timing of weather events, such as temperature changes and moisture accumulation. This has been shown to be a factor in cases of sudden death syndrome in soybean (Hershman et al. 1990; Wrather et al. 1995), as well as *Pythium* seedling blight of corn (Chen, 2016).

The CART analysis showed that crop rotation sequence was associated with a positive *X. vasicola* pv. *vasculorum* test in non-irrigated fields. Samples from non-irrigated fields with a continuous corn, corn-corn-soybean, or a corn-grain sorghum
rotation were more likely to test positive than samples from non-irrigated fields that rotated away from corn after one season (Figure 3). Multiple seasons of corn may cause a buildup of inoculum in infested corn residue, making disease development more likely. According to North et al. (1935), gumming disease was more difficult to control in New South Wales than in the tropics due to the common use of a two-year sugarcane crop rotation. A two-year corn rotation, such as the corn-corn-soybean rotation named in the CART tree as a predictor of disease, may have a similar effect on bacterial leaf streak management in corn. A corn-grain sorghum rotation was also a significant predictor of a positive test result, and was grouped with the continuous corn and corn-corn-soybean rotations in the CART analysis (Figure 3). This is intriguing, as Lang et al. (2017) demonstrated in a greenhouse setting that *X. vasicola* pv. *vasculorum* was capable of infecting sorghum, however infection has not been observed in sorghum fields near diseased corn fields in Nebraska or Kansas (Tamra Jackson-Ziems and Doug Jardine, personal communication, respectively).

Interestingly, non-irrigated fields that were rotated away from corn after one season, where minimum tillage, no-till, or strip tillage was used, only 24% of samples tested positive for *X. vasicola* pv. *vasculorum*. In contrast, in conventionally tilled fields, 67% of samples tested positive for the presence of *X. vasicola* pv. *vasculorum* (Figure 5). This seems counterintuitive if *X. vasicola* pv. *vasculorum* is capable of overwintering in corn residue like it is in sugarcane residue. Conventional tillage is expected to be more effective than minimal tillage methods (such as no till, strip till, and ridge till) at promoting degradation of residue, which can serve as an inoculum source (Peters et al.
The data obtained from the survey may be biased due to small sample sizes at this node. Only 9 respondents in this group used conventional tillage, whereas 33 respondents used a minimal tillage type.

Foxtail was the only alternative host of *X. vasicola pv. vasculorum* (Hartman et al. 2018) that was identified as a predictor of a positive *X. vasicola pv. vasculorum* test in the random forest analysis, and its presence in a field was not a strong predictor. However, not all reported weeds were examined in the random forest analysis, only weeds that were identified as problematic by more than 50% of respondents. Some respondents reported other alternative hosts of *X. vasicola pv. vasculorum* as problematic, including Johnsongrass and shattercane (Hartman et al. 2018), however there were not enough reports for these weeds to be examined with the random forest or chi-square analyses. Additionally, despite 7 prairie grasses being identified as alternative hosts for *X. vasicola pv. vasculorum*, CRP acres being directly adjacent to the field was also not a strong predictor of a positive *X. vasicola pv. vasculorum* test. It is possible that because *X. vasicola pv. vasculorum* infects these plants at such a low incidence in a field setting (Hartman et al. 2018), their presence does not increase the likelihood of disease development in corn.

Pre-plant nitrogen application was correlated with a positive *X. vasicola pv. vasculorum* test result according to the chi-squared analysis (Table 1). Research by Gent and Schwartz (2005) showed that while nitrogen fertilization had no effect on epiphytic populations of *X. axonopodis pv. allii* on onion, when high rates of nitrogen fertilizer were applied, symptom severity increased by up to 50% if environmental conditions were
favorable for the pathogen. Elings et al. (1997) found a similar effect when excessive nitrogen was applied to rice fields following infection with *Xanthomonas oryzae pv. oryzae*. It is unknown if respondents to this survey applied high rates of nitrogen in their preplant nitrogen application, as there were not enough responses to this question for it to be examined. The majority of survey responses were collected in the 2016 growing season, in which the environment was especially favorable for *X. vasciola pv. vasicola* in corn. A survey conducted in a season when the environment is less favorable for the pathogen may not find the same result. Effects of high nitrogen application rates have not been investigated for *X. vasicola pv. vasculum* in corn in a field setting, and further field experiments are needed to determine the effects of a preplant nitrogen application on bacterial leaf streak in corn.

Application of a herbicide containing glyphosate as an active ingredient was also favorable for samples testing positive for *X. vasicola pv. vasculum* (Table 2). Langemeier et al. (2017) found the same impact for *Clavibacter michiganensis subsp. nebraskensis* infection in corn. Glyphosate can directly impact plant susceptibility to diseases such as sudden death syndrome and root rot of soybean (Keen et al. 1982), root rot of barley (Smiley et al. 1992), and take-all of wheat (Hornby et al. 1998) by affecting the plant immune system. Inducible defenses such as the phenylpropanoid pathway (Dixon et al. 2002) and production of pathogenesis-related proteins (Hammond-Kosack and Jones, 2000) rely on the shikimic pathway or its products. The mechanism of action of glyphosate is to block the shikimic pathway, which may inhibit proper functioning of the plant immune system (Johal and Huber, 2009). Notably, only increased susceptibility
to soil-borne, root-infecting fungal and oomycete pathogens has been reported. Effects on bacterial leaf streak of corn, which is a foliar disease caused by a bacterial pathogen, may be different. As Langemeier et al. (2017) noted, reduced tillage is common when glyphosate is used as a means of weed control. Reduced tillage, which leaves infested residue on the soil surface and creates a source of inoculum, may be the main factor increasing the likelihood of pathogen presence, while glyphosate is only related to reduced tillage and is unrelated to pathogen presence. Additionally, both glyphosate and nitrogen are typically applied to more intensely managed fields, which are also more likely to be scouted for diseases such as bacterial leaf streak. Further research is needed to determine if disease development is affected by these factors.

This survey demonstrates that irrigation, planting date, crop rotation, and tillage are all agronomic practices that may affect bacterial leaf streak development in corn. These results indicate that certain agronomic practices used in Nebraska may have contributed to the outbreak and spread of *X. vasicola pv. vasculorum*. Based on the survey results, further research on bacterial leaf streak management should focus on early planting dates, reduced irrigation, crop rotation, and tillage as be ways to manage bacterial leaf streak. Further research is also needed to understand how environmental factors and residue cover influence disease development, and how irrigation can contribute to pathogen spread. Management recommendations should not be made based on these results until they are verified in future field and greenhouse studies.
Acknowledgements

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**Literature Cited**


Ishioka, T. eLmL 2013: The Fifth International Conference on Mobile, Hybrid, and Online Learning. pp 30-36.


Figure 1: Distribution of bacterial leaf streak in Nebraska corn during the 2016-2018 growing seasons. At least one sample received from each red county tested positive for *Xanthomonas vasicola* pv. *vasculorum* in a PCR test specific to the pathogen.
Figure 2: Area covered in corn residue in sampled fields, estimated by submitters of *Xanthomonas vasicola* pv. *vasculorum* positive corn samples on a scale of 1-100 (n=192).
Table 1: Five agronomic practices determined to be the most important predictors of a sample testing positive for *X. vasicola pv. vasculorum* based on results of the survey conducted during the 2016-2017 growing seasons.

<table>
<thead>
<tr>
<th>Agronomic Practice</th>
</tr>
</thead>
<tbody>
<tr>
<td>Irrigation</td>
</tr>
<tr>
<td>Growth Stage</td>
</tr>
<tr>
<td>Planting Date</td>
</tr>
<tr>
<td>Crop Rotation</td>
</tr>
<tr>
<td>Tillage</td>
</tr>
</tbody>
</table>
Figure 3: Classification tree generated by the CART analysis. Factors that appear at the top of the tree were determined to be the strongest predictors of samples testing positive for *Xanthomonas vasicola* pv. *vasculorum*.

Irrigation type: N=No irrigation; CP=Center pivot irrigation; GF=Gravity/flood; Ltrl=Lateral; SS=Sub Surface
Crop Rotation: CS=Corn-soybean; CSWS=Corn-soybean-wheat-soybean; WCF=Wheat-corn-fallow; CC=continuous corn; CCB=Corn-corn-soybean; CM=corn-grain sorghum
Growth Stage: V79=V7-V9; 10T=V10-VT; R1=R1-R3; R4=VE-V7 or R4-R6
Planting Date: G1=Before April 21st; G2=April 22nd-May1st; G3=May 2nd-May 12th; G4=May 13th-May 23rd; G5=After May 24th
Tillage Type: M=Minimum till; N=No till; S=Strip Till; C=Conventional till
Figure 4: Ranking of variable importance per the Random Forest analysis. Agronomic and environmental factors with higher gini coefficients were determined to be more strongly associated with disease development.
Table 2: Chi-square analysis of correlations between fertilizer application and sample test results for *Xanthomonas vasicola* pv. *vasculorum*. $Xvv$ is an abbreviation for *X. vasicola* pv. *vasculorum*. Variables were tested at an alpha level of 0.05.

<table>
<thead>
<tr>
<th>Fertilizer Application</th>
<th>Xvv +</th>
<th>Xvv -</th>
<th>p-value</th>
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<tr>
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<tr>
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<td></td>
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<td><strong>Pre-Plant Potassium</strong></td>
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<td></td>
</tr>
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<tr>
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</tr>
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<td><strong>Pre-Plant Phosphorus</strong></td>
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Table 3: Chi-square analysis of correlations between pesticide application and sample test results for *Xanthomonas vasicola* pv. *vasculorum*. Xvv is an abbreviation for *X. vasicola* pv. *vasculorum*. Variables were tested at an alpha level of 0.05.

<table>
<thead>
<tr>
<th>Pesticide Active Ingredient</th>
<th>Xvv vasicola pv. vascurom test result (%)</th>
<th>p-value</th>
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<tbody>
<tr>
<td></td>
<td>Xvv +</td>
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<td>Acetochlor</td>
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<tr>
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</tr>
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<td>Fungicide</td>
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<td>n=145</td>
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Table 4: Chi-square analysis of correlations between soil types and sample test results for *Xanthomonas vasicola* pv. *vasculorum*. *Xvv* is an abbreviation for *X. vasicola* pv. *vasculorum*. Variables were tested at an alpha level of 0.05.

<table>
<thead>
<tr>
<th>Soil Type</th>
<th>Xanthomonas vasicola pv. vasculorum test result (%)</th>
<th>p-value</th>
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<tr>
<td></td>
<td><strong>Xvv +</strong></td>
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APPENDIX
INVESTIGATION OF IN VITRO INHIBITION OF XANTHOMONAS VASICOLA PV. VASCULORUM GROWTH BY PESTICIDES AND COPPER FERTILIZERS

Abstract

Xanthomonas vasicola pv. vasculorum was reported on Nebraska corn samples for the first time in the United States in 2016. Symptoms caused by the pathogen include water-soaked, yellow or tan to dark brown interveinal streaks with wavy margins. This pathogen also causes disease in sugarcane, but can be controlled in sugarcane with resistant hybrids. Corn hybrids with resistance to X. vasicola pv. vasculorum are not yet commercially available, therefore other methods must be used to manage the disease. The objective of this experiment was to evaluate potential inhibition of X. vasicola pv. vasculorum growth by certain products. In modified disk diffusion assays, a culture of X. vasicola pv. vasculorum was spread on nutrient broth yeast extract (NBY) agar plates, and disks soaked in Badge, Cop-R-Quik, Max-In Copper, pyraclostrobin, Domark, or Actinovate were placed on each of the plates. Inhibition of bacterial growth was quantified by measuring the distance from the edge of the disk to the edge of the bacterial growth. In a second experiment, liquid NBY media was amended with Badge, Cop-R-Quik, Max-In Copper, pyraclostrobin, or Domark, and inoculated with X. vasicola pv. vasculorum. After 24 hours of growth, bacterial colony forming units were estimated with the 8-spot method. Zones of inhibition varied between treatments (p<0.0001), with Cop-R-Quik having the largest zone of inhibition. Badge and Domark had smaller zones of inhibition than Cop-R Quik, but larger zones of inhibition than Actinovate, pyraclostrobin, and Max-In Copper. Actinovate, pyraclostrobin, and Max-In Copper had
the smallest zones of inhibition. Final CFU/ml of *X. vasicola pv. vasculorum* in liquid media amended with the products also varied among treatments. Final bacterial concentrations were: $10^{10}$ CFU/mL in the untreated control, $10^3$ CFU/mL in media amended with Badge, $10^{10}$ CFU/mL in media amended with Cop-R-Quik, $10^8$ CFU/mL in media amended with Domark, $10^8$ CFU/mL in media amended with pyraclostrobin, and $10^{10}$ CFU/mL in media amended with Manzate. Products performed differently in different tests, however some of these product may provide some bacterial control.

**Introduction**

*X. vasicola pv. vasculorum* is a foliar, bacterial corn pathogen that causes water soaked, yellow or tan to dark brown interveinal streaks on leaves. This pathogen was first reported in the United States in 2016 on Nebraska corn samples (Korus et al. 2017). Management techniques for *X. vasicola pv. vasculorum* in corn are not well understood. *X. vasicola pv. vasculorum* is also the causal agent of gumming disease of sugarcane (North et al. 1935), where management of the pathogen has been more thoroughly studied. In sugarcane, the pathogen is managed primarily with resistant hybrids (North, 1935; Ricaud and Autrey, 1989). *X. vasicola pv. vasculorum* resistant corn hybrids are currently unavailable, therefore corn growers must rely on other disease management strategies whose efficacy has yet to be proven. Management strategies that have been effective for some other residue-borne pathogens, such as crop rotation to a non-host crop, tillage to bury and increase the degradation of infested residue, or use of chemical control methods are currently under evaluation.
Disk diffusion assays are in vitro laboratory tests used to challenge the growth of pathogens with various treatments. The techniques involve exposing a bacterium, such as *X. vasicola pv. vasculorum*, to an antibiotic (or other compound of interest) via small filter paper disks treated with the compound of interest. When placed on an agar plate with a culture of the bacterium spread across the entire plate, the compound diffuses away from the disk. This creates a concentration gradient of the compound, with the highest concentration being the area immediately around the disk. The bacteria grow toward the disk until they reach a concentration that is too high, where growth is inhibited. The area around the disk where the bacterium is unable to grow is referred to as the zone of inhibition, and resembles a clear area or halo surrounding the disk. The width of the cleared area is used to quantitatively measure the efficacy of a compound versus a bacterium (Tendencia, 2004; Schlund, 2015).

Numerous copper-based fungicides/bactericides are commercially available for disease management. Although they are often labeled as fungicides, these products can be effective at controlling bacterial pathogens as well. These products are contact pesticides, which remain on the leaf surface and are not absorbed or moved into the plant like systemic products. Contact pesticides on the leaf surface are vulnerable to being washed off the plant by rainfall or irrigation, often requiring multiple applications and good application coverage for effective disease control (Kennelly et al. 2007). Schwartz and Otto (1998) demonstrated that when amended with an ethylenebisdithiocarbamate (EBDC) fungicide, copper bactericides inhibited development of Xanthomonas leaf blight of onion, caused by *Xanthomonas axonopodis pv. allii*, however multiple
applications were required for disease control, and it was necessary to make applications preventively (Marco and Stall, 1983). Field studies have also been conducted to evaluate the efficacy of copper products against Goss’s bacterial wilt and blight in corn, with inconsistent results. Results from research conducted by Oser et al. (2013) and Wise et al. (2014) showed no reduction in disease severity when an application of copper hydroxide was made under very low disease severity. However, Korus et al. (2010) observed a reduction in disease severity when an application of copper hydroxide was made during the seedling corn stages prior to inoculation. These products have not been evaluated for efficacy against *X. vasicola* pv. *vasculorum*.

Copper-based fertilizers are commercially available and used in some higher value crops. The fertilizers are not labeled as pesticides, and cannot legally be applied specifically for disease control. However, according to Poschenrieder et al. (2006), metals like copper have bacteriostatic properties, and can therefore reduce pathogen populations on the surface of a plant. Additionally, an experiment by Dutta et al. (2017) noted a correlation between the SAR immune response and the concentration of copper in the soil, in addition to a decrease in bacterial leaf spot severity in pepper when copper concentrations within the fruit increased.

Biological control involves the introduction of an organism (or biological control agent) that is antagonistic or competitive with the pathogen by one or more mechanisms. This organism may compete with the pathogen for nutrients or space (Elad and Chet, 1987). This mechanism can be effective if the pathogen has a long epiphytic period, or requires nutrients on plant surfaces. The organism may also actively kill the pathogen via
production of antimicrobial compounds, such as the volatile organic compounds produced by certain species of *Bacillus* (Massawe et al. 2018), or by parasitism, such as *Pythium oligandrum* infection of *Verticillium dahliae*, causal agent of Verticillium wilt of pepper and other crops. Organisms such as *Lysobacter enzymogenes* can elicit an immune response in the plant, preemptively creating a defense against pathogens (Kilic-Ekici and Yuen, 2003). It should be noted that in order for these biological control agents to effectively control a pathogen, the environment must be conducive to the growth of the biological control agent (Burpee, 1989; Hannusch and Boland, 1996), and the use of multiple biological control agents with different mechanisms of action and different environmental needs may be necessary for adequate disease control (Guetsky et al. 2001; Guetsky et al. 2002).

None of these or similar products have been evaluated for efficacy against *X. vasicola pv. vasculorum*. The objective of this experiment was to determine the potential for fungicidal and nutritional coppers and biological control agents to inhibit *X. vasicola pv. vasculorum* growth *in vitro* via disk diffusion assays and to examine the growth of the pathogen in media amended with one of the products of interest, compared to media that was not amended with one of the products. Pathogen control *in planta* and in a natural field setting was not evaluated in this experiment.

**Materials and Methods**

**Strain Preparation**

*X. vasicola pv. vasculorum* strain 2016000401X was obtained from corn leaves from a naturally-infested field in Clay County, NE in 2016 and confirmed as *X. vasicola*
pv. *vasculorum* with a PCR assay developed by Lang et al. (2017). The strain was stored at -80°C in Microbank storage vials (Pro-Lab Diagnostics Inc., Toronto, Canada) containing beads and a proprietary storage solution (Langemeier et al. 2014). The strain was removed from storage for use in the experiment by placing a single bead on a nutrient broth yeast extract (NBY) plate (Vidaver et al. 1967) and the plate was streaked to obtain single colonies. The plate was incubated at 27°C for 48 hours.

*Disk diffusion assays*

This experiment was arranged in a completely randomized block design, blocked by plate and repeated once in time. Ethanol was used as a positive control, and sterile water was used as a negative control. Chemicals used in this experiment were diluted to concentrations that would be sprayed in the field, according to each product’s label (Table 1). A single, isolated colony of strain 201600401X was picked from an NBY plate and placed in a 200 mL flask with 100 mL of liquid NBY media. The flask was shaken at and maintained at 27°C for 48 hours. A bacterial concentration of c.a. 10⁹ CFU/mL was estimated with serial dilutions in each experiment. Five hundred µl of *X. vasicola pv. vasculorum* suspended in liquid NBY was pipetted onto each NBY plate and spread with the spread plate technique by Prabuseenivasan et al. (2006). Plates were dried in a sterile fume hood for a total of 4 hours, 3 hours with the lids completely closed and 1 hour with the lids slightly offset. Sterile filter paper disks, 7 mm in diameter, were each soaked in one product, and one disk per product was placed on each plate. After incubation at 27°C for 48 hours, zones of inhibition were measured from the edge of the disk to the edge of the bacterial growth with a caliper.
Liquid media amendment with fungicides and nutritional coppers

This experiment was a completely randomized design, and was conducted three times, with five replicates of each treatment. Chemicals used in this experiment were diluted to concentrations that would be sprayed in a field (Table 1). An untreated culture of *X. vasicola* pv. *vasculorum* was included as a positive control to ensure that bacterial growth was not inhibited by the conditions under which the bacteria were maintained or the methods used other than treatments. Liquid media not inoculated with *X. vasicola* pv. *vasculorum* was included as an additional control to ensure that bacterial concentrations were not affected by contamination during treatment or maintenance of the cultures. Liquid NBY was pipetted into tubes at an amount of 9.8 mL, followed by 100 µl each of one treatment chemical and c.a. $10^9$ CFU/mL *X. vasicola* pv. *vasculorum* suspended in liquid NBY. Tubes were maintained at 27°C for 24 hours. Bacterial concentration was quantified with the 8-spot cell enumeration technique (Harris and Sommers, 1967)

**Statistics**

Statistical analyses were conducted in R version 3.4.0. A one-way ANOVA was used to examine treatment differences in inhibition of *X. vasicola* pv. *vasculorum* growth ($\alpha=0.05$).

**Results**

**Disk Diffusion Assays**

In the disk diffusion assays, there were no differences in inhibition between chemical treatments on different plates ($p=0.999$). There were also no differences in treatment effects between the two repeats of this experiment ($p=0.897$). Data from all
plates and both repeats were combined for analysis due to the lack of an effect on the zones of inhibition. Inhibition of bacterial growth varied across treatments (p<0.0001). Ethanol, the positive control, showed the greatest zone of inhibition at 2.494 mm. The negative control, sterile water, did not inhibit bacterial growth, with a zone of inhibition of 0 mm. This demonstrates that there was no effect on inhibition due to the sterile water in which the products were diluted. Products containing copper as an active ingredient inhibited bacterial growth, with Max-in copper being the weakest inhibitor (average 0.022 mm zone of inhibition) and Cop-R-Quik being the strongest inhibitor with an average 1.280 zone of inhibition (Table 2). Domark, a fungicide containing the active ingredient tetraconazole, inhibited bacterial growth with an average zone of inhibition of 0.352 mm. Copper hydroxide-based fungicide Badge also created a small zone of inhibition, with an average zone of 0.460 mm. Headline, a fungicide containing pyraclostrobin, did not inhibit bacterial growth, with an average zone of inhibition of 0 mm. Biological control agent Actinovate also showed no inhibition of bacterial growth, with an average zone of inhibition of 0 mm (Table 2).

Media Amendments

No contamination was observed in the non-inoculated liquid NBY. In the experiment examining liquid media amended with fungicides and nutritional coppers, there were no differences in treatment effects on bacterial concentrations between the three repeated experiments (p=0.8841). Due to the lack of differences, data from the three repetitions were combined for analysis. Treatment differences in the inhibition of bacterial growth were observed (p<0.0001), although not every product inhibited growth.
There were no differences observed between Badge, Cop-R-Quik, Domark, and pyraclostrobin, however when final bacterial concentrations were compared with the non-treated control these treatments inhibited bacterial growth. The final bacterial concentration in the Manzate treatment was not different from the non-treated control (Table 3).

**Discussion**

Disk diffusion assays, while useful for testing microbial sensitivity to and efficacy of certain products, are not optimal for testing the efficacy of agricultural chemicals. These assays were originally developed to test bacterial sensitivity to antibiotics, and later adapted to test the efficacy of essential oils against certain bacteria (Prabuseenivasan et al. 2006). These assays depend on a product’s ability to diffuse through the agar. This method works well for antibiotics and essential oils, which are made of small molecules, however the larger molecules of fungicides, nutritional coppers, and other agricultural chemicals may not diffuse through the agar as well. This could lead to inaccurate measures of a product’s ability to control bacterial growth. For this reason, products were also evaluated in liquid media amended with the different products.

Each experimental product tested in both the disk diffusion assays and as liquid media amendments showed some inhibition in both experiments (Tables 2, 3), with the exception of pyraclostrobin, which inhibited bacterial growth when used as a liquid media amendment, but not in the disk diffusion assays. However, unlike the results from the disk diffusion assays, there was no separation between the performance of each product against *X. vasicola* pv. *vasculorum* in the liquid media amendment experiment.
Bacterial concentrations in the amended media were estimated with the 8-spot method, a method that does not precisely estimate bacterial population densities (Harris and Sommers, 1967). The use of the 8-spot cell enumeration method may account for the lack of separation of final bacterial concentrations between products.

Bacterial growth in liquid media amended with Manzate was no different from bacterial growth in non-amended media (Table 3). Mancozeb, the active ingredient in Manzate contains an Ethylenebisdithiocarbamate (EBDC), Manganese, and Zinc complex. EDBC is a fungicide, but it is mixed with copper and used in the control of Xanthomonas leaf blight of onion in Colorado (Schwartz and Otto, 1998). Manganese is not a bactericide, however manganese requirements in fungal and bacterial pathogens are much lower than in plants, which can be exploited by pathogens. Zinc has a demonstrated toxicity to bacteria and other plant pathogens (Graham and Webb, 1991; Raghupathi et al. 2011), however zinc comprises only a small part of the active ingredient complex of Mancozeb. Bacterial control in Manzate amended media may be improved if a larger proportion of the active ingredients in Manzate were bactericidal.

The results of disk diffusion assays indicated that copper-based fertilizers can control the growth of X. vasicola pv. vasculorum, however Cop-R-Quik created a larger zone of inhibition than Max-In Copper, in fact Cop-R-Quik had the largest zone of inhibition of any product tested with the exception of Ethanol, which was the positive control (Table 2). This may be due to the different formulation of Cop-R-Quik, which is amino acid complexed, with improved solubility over other nutritional coppers (US008529964B1 September 10, 2013). Disk diffusion assays rely on a product’s ability
to diffuse through the agar (Prabuseenivasan et al. 2006), and diffusion of Cop-R-Quik may be superior to that of Max-In Copper due to the improved solubility of Cop-R-Quik.

Fungicides Badge and Domark both showed some control of *X. vasicola* pv. *vasculorum* in the disk diffusion assays, while pyraclostrobin showed no inhibition of *X. vasicola* pv. *vasculorum* growth in these assays (Table 2). Badge contains copper as an active ingredient, which has been shown to have bactericidal effects when applied for control of bacterial diseases in some pathosystems, such as Xanthomonas leaf blight of onion (Schwartz and Otto 1998), but not all pathosystems, as Wise et al. (2014) and Oser et al. (2013) found no control of *Clavibacter michiganensis* subsp. *nebraskensis* in corn. This suggests that while copper based fungicides can inhibit bacterial growth in a controlled lab setting, the host and environment also play a role in the efficacy of copper-based fungicides in the field. Neither Domark nor pyraclostrobin is a copper-based fungicide, however Domark showed some control of bacterial growth in the disk diffusion assay, and pyraclostrobin showed some control of bacterial growth when used as a medium amendment (Tables 2,3). Although these fungicides contain different active ingredients, neither product contains an active ingredient with known bactericidal properties. This indicates that inhibition of bacterial growth may be due to components in the inert ingredients that are not disclosed. For example, some products contain proprietary mixes that include alcohol (Schlund, 2015), which may be added to the fungicides to improve efficacy.

Actinovate showed no inhibition of bacterial growth in the disk diffusion assays (Table 2). The biological control agent in Actinovate is *Streptomyces lydicus*, which
controls pathogenic fungi by producing antifungal metabolites (Yuan and Crawford, 1995). Given that the biological control agent in this product had no known bactericidal properties, it is likely that this particular organism does not produce antibacterial compounds, hence its lack of control of *X. vasicola pv. vasculorum*. Other products containing biological control agents may show more efficacy against *X. vasicola pv. vasculorum*, however their mechanism of action must be considered to properly test them.

In conclusion, there are some products, namely Cop-R-Quik, Badge, and Domark, that consistently inhibited the growth of *X. vasicola pv. vasculorum* in both experiments. While these products inhibited *X. vasicola pv. vasculorum* growth *in vitro*, their efficacy when applied to corn in field settings must also be evaluated in order to make effective disease management recommendations. Further research is also needed to determine if the pathogen is controlled by the active ingredients, or if the adjuvants contain ingredients with antibacterial properties that inhibit pathogen growth.
Literature Cited


Table 1: Products tested for inhibition of *Xanthomonas vasicola* pv. *vasculorum* growth.

<table>
<thead>
<tr>
<th>Trade Name</th>
<th>Chemical Class</th>
<th>Active Ingredient</th>
<th>Concentration</th>
<th>Experiment</th>
<th>Manufacturer</th>
</tr>
</thead>
<tbody>
<tr>
<td>Actinovate</td>
<td>Biological</td>
<td><em>Streptomyces lydicus</em></td>
<td>0.2% v/v</td>
<td>Disk diffusion assay</td>
<td>Valent Biosciences, LLC, Libertyville, IL</td>
</tr>
<tr>
<td>Badge</td>
<td>Fungicide</td>
<td>Copper Hydroxide; Copper Oxychloride</td>
<td>10% v/v</td>
<td>Disk diffusion assay; Media amendment</td>
<td>Gowan Company, Yuma, AZ</td>
</tr>
<tr>
<td>Cop-R-Quik</td>
<td>Nutritional Copper</td>
<td>Copper Nitrate</td>
<td>3.1% v/v</td>
<td>Disk diffusion assay; Media amendment</td>
<td>Natural Ag Solutions, LLC, Sebring, FL</td>
</tr>
<tr>
<td>Domark</td>
<td>Fungicide</td>
<td>Tetraconazole</td>
<td>2.3% v/v</td>
<td>Disk diffusion assay; Media amendment</td>
<td>Gowan Company, Yuma, AZ</td>
</tr>
<tr>
<td>Headline</td>
<td>Fungicide</td>
<td>Pyraclostrobin</td>
<td>2.8% v/v</td>
<td>Disk diffusion assay; Media amendment</td>
<td>BASF Corporation, Research Triangle Park, NC</td>
</tr>
<tr>
<td>Manzate</td>
<td>Fungicide</td>
<td>Zinc ion and Manganese Ethylenebisdithiocarbamate</td>
<td>2.4% v/v</td>
<td>Disk diffusion assay</td>
<td>United Phosphorus, Inc., King of Prussia, PA</td>
</tr>
<tr>
<td>Max-In Copper</td>
<td>Nutritional Copper</td>
<td>Copper</td>
<td>2.6% v/v</td>
<td>Disk diffusion assay; Media amendment</td>
<td>Winfield Solutions, LLC, St. Paul, MN</td>
</tr>
</tbody>
</table>
Table 2: Inhibition of Xanthomonas vasicola pv. vasculorum growth in disk diffusion assays. Zone of inhibition is the distance between the edge of the treated disk and the edge of X. vasicola pv. vasculorum growth. Letters denote significance within a column at $\alpha=0.05$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Zone of Inhibition (mm)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Untreated</td>
<td>0±0 a</td>
</tr>
<tr>
<td>Actinovate</td>
<td>0±0 a</td>
</tr>
<tr>
<td>Headline</td>
<td>0±0 a</td>
</tr>
<tr>
<td>Max-In Copper</td>
<td>0.022±0.644 a</td>
</tr>
<tr>
<td>Badge</td>
<td>0.460±0.274 b</td>
</tr>
<tr>
<td>Domark</td>
<td>0.352±0.296 b</td>
</tr>
<tr>
<td>Cop-R-Quik</td>
<td>1.280±0.477 c</td>
</tr>
<tr>
<td>Ethanol</td>
<td>2.494±0.644 d</td>
</tr>
</tbody>
</table>
Table 3: Concentrations of *Xanthomonas vasicola* pv. *vasculorum* in liquid nutrient broth yeast extract media amended with a fungicide or a nutritional copper. Letters denote significance within a column at $\alpha=0.05$.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Estimated final <em>X. vasicola</em> pv. <em>vasculorum</em> Concentration (CFU/mL)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Negative Control</td>
<td>0 a</td>
</tr>
<tr>
<td>Badge</td>
<td>$10^3$ b</td>
</tr>
<tr>
<td>Domark</td>
<td>$10^8$ b</td>
</tr>
<tr>
<td>Headline</td>
<td>$10^8$ b</td>
</tr>
<tr>
<td>Cop-R-Quik</td>
<td>$10^{10}$ bc</td>
</tr>
<tr>
<td>Manzate</td>
<td>$10^{10}$ c</td>
</tr>
<tr>
<td>Positive Control</td>
<td>$10^{10}$ c</td>
</tr>
</tbody>
</table>