Infection, Survival, and Growth of *Clavibacter nebraskensis* on Crop, Weed, and Prairie Plant Species

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Abstract

Clavibacter nebraskensis is the causal agent of Goss's leaf blight and wilt, an important disease of maize in the United States and Canada. The epidemiology and ecology of this bacterial pathogen are poorly understood. Infested maize residue is often considered to be the primary source of inoculum for maize; however, the potential for many other plant species to be infected and serve as inoculum sources is unknown. The goal of this study was to determine if *C. nebraskensis* could infect, survive, and grow on common weed, crop, and grass species. Seedling leaves of 18 plant species that grow in maize production areas in the United States were inoculated with this pathogen in a controlled environment and in the field. Lesion development, bacterial streaming, and pathogen population size on leaves were then determined and used as criteria to evaluate host–pathogen interactions. Woolly cupgrass (*Eriochloa villosa*) and the native prairie grasses big bluestem (*Andropogon gerardii*)

and little bluestem (*Schizachyrium scoparium*) developed lesions and bacterial streaming, identifying them as hosts and susceptible to infection. To our knowledge, this is the first report of these grass species being hosts of *C. nebraskensis*. Ten other grass species, including wheat and oats, were identified as potential sustaining hosts that maintained epiphytic or endophytic pathogen populations >10⁶ colony forming units per leaf sample but displayed no evidence of infection. Five broadleaf species tested were nonhosts based on the three criteria. This study suggests that multiple plant species support infection and growth of *C. nebraskensis* and further elucidates the ecology of this pathogen and the epidemiology of Goss's wilt.

Keywords: bacterial, pathogen, prokaryotes, field crops, cereals and grains, epidemiology

The gram-positive bacterial pathogen *Clavibacter nebraskensis* (Vidaver and Mandel 1974) Davis et al. 1984, Li et al. 2018, comb. nov. is the causal agent of Goss's leaf blight and wilt. This disease, commonly referred to as Goss's wilt, is a significant disease of maize (*Zea mays* L.) that can reduce grain yield more than 50% (Jackson et al. 2007; Jardine and Claflin 2016). Leaf blight is the most common result of infection by *C. nebraskensis*, although vascular wilt can also develop. The ability of this pathogen to infect and damage many plants other than maize has not been well understood.

Goss's wilt of maize was first reported in Nebraska in 1969; and it subsequently became problematic in adjoining states (Jackson et al. 2007; Schuster 1972). Within a decade, however, incidences of Goss's wilt had subsided to low levels in that region (Jackson et al. 2007; Wysong et al. 1981). Beginning in 2006, Goss's wilt reemerged as a spreading and significant problem in many areas in the United States (Jackson et al. 2007). This disease has been confirmed as far north as northern Minnesota in the United States and Manitoba and Alberta in Canada, south to Texas and Louisiana, west to Colorado, and east to Indiana (Friskop et al. 2014; Harding et al. 2018; Howard et al. 2015; Korus et al. 2011; Malvick et al. 2010; Ruhl et al. 2009; Singh et al. 2015; Vidaver et al. 1981). Although maize production occurs over a larger area in North America than the area where Goss's wilt has been reported, this disease has generally remained restricted to the region once occupied by the native tall and mixed grass prairies that extended from the Canadian Prairie

provinces to Mexico and from the Rocky Mountains to Indiana (Samson and Knopf 1994).

The origin of Goss's wilt and *C. nebraskensis* and the reasons for its reemergence and spread since the mid-2000s are poorly understood. In localized disease outbreaks, infection and disease development often occur after strong summer storms and hail (Jardine and Claflin 2016). Disease outbreaks are also associated with the planting of hybrids susceptible to Goss's wilt, continuous maize cropping practices, high plant populations, and persistent maize residue cover due to reduced tillage (Jackson et al. 2007; Langemeier et al. 2017). These factors are not associated with disease in all situations, however, such as in fields without recent maize production, suggesting that additional factors may be important.

Critical factors relating to the development of Goss's wilt likely include the source of inoculum and the habitats used by the pathogen when maize residue is not present. Infested maize residue from previous crops is typically considered the primary source of inoculum for maize (Eggenberger et al. 2016; Schuster 1975). *C. nebraskensis* can disseminate from point sources of inoculum, such as infested maize residue, and initiate disease in maize production fields (Eggenberger et al. 2016). When Goss's wilt develops in fields without infested maize residue, however, other nearby inoculum sources are thought to play an important role. Infested seed appears unlikely to contribute enough inoculum to initiate widespread disease in fields in a single season (Block et al. 2019). Plants and residue other than maize that support epiphytic or endophytic populations of the pathogen might play important roles in the Goss's wilt pathosystem by acting as inoculum reservoirs. For other bacterial diseases, including some caused by *Clavibacter* spp., *Pseudomonas* spp., and *Xanthomonas* spp., grassy and broadleaf weeds have been reported to serve as likely reservoirs of inoculum (Barak et al. 2001; Hirano and Upper 1983; Latorre and Jones 1979; Schaad and Dianese 1981; Van Der Wolf et al. 2005).

Multiple plant species in the Family Poaceae are potential sources of inoculum for *C. nebraskensis*. Eastern gamagrass (*Tripsacum dactyloides* L.), grain sorghum (*Sorghum vulgare* L.), shattercane (*Sorghum bicolor* L.) Moench subsp. *arundinaceum*, sugarcane (*Saccharum officinarum* L.), sudangrass (*Sorghum bicolor* L.) Moench subsp. *drummondii*, and teosinte (*Euchlaena mexicana* Schrad.) were

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reported by Schuster (1975) to be hosts that develop symptoms following inoculation with *C. nebraskensis*. In addition, green foxtail (*Setaria viridis*), bristly foxtail (*S. verticillata*), yellow foxtail (*S. pumila*), and giant foxtail (*S. faberi*), annual ryegrass (*Lolium multiflorum* Lam), Johnson grass (*Sorghum halepense* (L.) Pers.), and large crabgrass (*Digitaria sanguinalis* (L.) Scop.) have been reported to be hosts of *C. nebraskensis* based on symptom development in inoculated plants (Ikley et al. 2015; Langemeier et al. 2014). This pathogen was isolated from barnyard grass (*Echinochloa crus-galli* (L.) Beauv.); however, subsequent experiments failed to confirm that it can be infected (Schuster 1972; Wysong et al. 1981). Given the diversity of weed, crop, and cover crop species that have not been evaluated as potential hosts of *C. nebraskensis*, the list of known hosts is likely incomplete. To our knowledge, many grasses and broadleaf species have not been evaluated for their potential to act as hosts or to maintain epiphytic or endophytic populations of *C. nebraskensis*.

The goal of this study was to determine if various plant species common in maize production areas in the Upper Midwest can be infected or colonized by *C. nebraskensis* and potentially serve as inoculum reservoirs. The specific objectives were: 1) to determine the reaction of common weeds, crops, cover crops, and native grasses in maize producing areas to inoculation with *C. nebraskensis*; and 2) to determine if *C. nebraskensis* is able to survive and multiply epiphytically on the tested plant species.

Materials and Methods

Bacterial isolate and inoculum preparation. *C. nebraskensis* isolate CIC251 was used for all experiments in this study. This isolate originated from an infected maize leaf collected in Minnesota and was confirmed as *C. nebraskensis* by 16S rDNA sequencing and with Koch's postulates (Malvick et al. 2010). For the experiments, it was grown on nutrient broth yeast agar (NBY) (Vidaver 1967) supplemented with 0.1 g/ml cyclohexamide for 5 days at 25°C. Single colonies were selected, spread on NBY, and incubated at 25°C for 3 days. Bacteria were scraped from the plates, suspended in 0.85% NaCl, and adjusted spectrophotometrically to approx. 1×10^8 CFU/ml.

Plant species and seed sources. Eighteen plant species in four categories were included in this study. The categories were (i) high acreage crop plants in the upper midwestern United States, (ii) commonly planted cover crop species, (iii) weed species that are common in or near midwestern maize fields, and (iv) native grasses from midwestern prairies. Soybean (*Glycine max*, cv. MN 1410), spring wheat (*Triticum aestivum* L.), oats (*Avena sativa*), barley (*Hordeum vulgare* L.), and sunflower (*Helianthus annuus*) (Mycogen Seeds, Indianapolis, IN) represented high acreage crops. Common waterhemp (*Amaranthus tuberculatus*), common ragweed (*Ambrosia artemisiifolia*), pigweed (*Amaranthus retroflexus*), woolly cupgrass (*Eriochloa villosa*), smooth brome grass (*Bromus inermis*), large crabgrass (*Digitaria sanguinalis*), proso millet (*Panicum miliaceum*), witchgrass (*P. capillare*), fall panicum (*P. dichotomiflorum*), quackgrass (*Elytrigia repens*), and reed canarygrass (*Phalaris arundinacea*) represented common weeds (seed sourced from B. Kinkaid, University of Minnesota). Winter cereal rye (*Secale cereale* L.) and hairy vetch (*Vicia villosa*) (Johnny's Selected Seeds, Fairfield, ME), buckwheat (*Fagopyrum esculentum*), and annual ryegrass (*Lolium multiflorum* Lam.) represented common cover crops. Big bluestem (*Andropogon gerardii*) and little bluestem (*Schizachyrium scoparium*) represented common native grasses of the midwestern prairies (Prairie Moon Nursery, Winona, MN). Maize hybrid Mo1 (sourced in 2012 from Monsanto Corp.) and teosinte (sourced from the USDA Germplasm Resource Information Network, Ames, IA) were included as susceptible checks.

Greenhouse studies of host range and colonization. Seeds of all plant species were sown in LC-8 potting mix (Sungro Horticulture, Agawam, MA) in 8" square pots, maintained in a greenhouse with a 14-h photoperiod at 24°C, watered 4 times/week, and fertilized weekly. Leaves in each pot were inoculated with suspensions of *C. nebraskensis* after 4 weeks of growth for all species except maize (grown 3 weeks) using a pinprick wounding device (Blanco et al.

1977; Chang et al. 1977). The wounding portion of the device was 2 cm in diameter and contained 37 sharp pins. Controls were mock-inoculated using 0.85% NaCl in place of bacterial suspensions. Two replicated studies were conducted at different times, and each study included two inoculated and two noninoculated replications in different pots arranged in a completely randomized design.

Inoculated leaves were assessed 3 weeks postinoculation (WPI) for the presence of bacterial streaming, necrosis, water-soaking and freckling, and leaf reddening. Bacterial streaming was assessed by placing cut leaf pieces on a glass microscope slide, covering with a drop of sterile H₂O, and examining cut edges microscopically at 400×. Disease severity was rated within and beyond the 2-cm-diameter inoculation area using a 0–5 rating scale modified from Milus and Mirlolhi (1994). For this scale, 0 = no visible symptoms beyond pinprick wounds, 1 = reddening or chlorosis but no water-soaking or necrosis, 2 = chlorosis and reddening with water-soaking, necrosis, and freckling less than 25% of inoculated area, 3 = chlorosis and reddening with water-soaking, necrosis, and freckling 25 to 100%, 4 = chlorosis and reddening with water-soaking, necrosis, and freckling extending up to 1 cm from edge of inoculated area, and 5 = chlorosis and reddening with water-soaking, necrosis, and freckling extending more than 1 cm from inoculated area. Leaves were considered as infected when necrosis, water-soaking, freckling, leaf reddening, and bacterial streaming developed, and the mock-inoculated control plants of the same species developed no symptoms or streaming. Disease severity data were analyzed with JMP Pro13 software (SAS Institute Inc., Cary, NC). The Wilcoxon method revealed no significant differences between the two replicated experiments ($P = 0.05$), and data from both experiments were combined for analysis. Differences among mean severity values were determined using the Kruskal-Wallis test, and nonparametric comparisons for each pair of plant species were determined using the Wilcoxon method ($P = 0.05$).

Greenhouse studies of leaf colonization by *C. nebraskensis*. Populations of *C. nebraskensis* on inoculated leaves 3 WPI were determined by placing 2 cm² samples of tissue from inoculated leaves in a sterile aqueous solution of 0.85% NaCl, vortexing for 5 s, soaking for 3 min, and spreading serial dilutions on NBY medium. The leaf tissues for this analysis were taken either from the margins of lesions or from the inoculation site if no lesions developed. After 5–7 days of incubation at 25°C, colonies typical of *C. nebraskensis* were counted (Vidaver and Mandel 1974). Identity of a subset of the isolates was also assessed using Agdia ImmunoStrip for *Clavibacter michiganensis* subsp. *michiganensis* (Agdia Inc., Elkhart, IN), which cross-reacts with *C. nebraskensis*. Differences among log₁₀ transformed CFU values measured on each plant species were determined as described above ($P = 0.05$).

Field studies of infection and survival. Two replicated studies with 18 plant species each were conducted to evaluate the host range of *C. nebraskensis* in field environments. One study was located in Falcon Heights, MN and the other in Rosemount, MN. Each study contained three replicates per plant species for each of two inoculation methods arranged in a randomized complete block design. Seed was sown in early June. The fastest growing species (maize, soybean, winter cereal rye, sunflower, barley, spring wheat, buckwheat, annual ryegrass, and proso millet) were inoculated in early July, and others were inoculated in late July. Plants were inoculated with each of two methods: a pinprick wounding device as described above and by spraying (Solo 451 Mist Blower, Solo USA, Newport News, VA) with suspensions of *C. nebraskensis*, prepared as described above and containing 1 g/liter of silicon carbide (Fischer Chemical). Control plants for both methods were mock inoculated with a sterile aqueous solution of 0.85% NaCl and silicon carbide. Plants were assessed weekly for symptom development. Leaves with lesions or symptoms of bacterial infection were collected and bacteria were isolated and serially diluted as described above. Five colonies per dilution series were selected arbitrarily and characterized for Gram reaction using the KOH method (Halebian et al. 1981) and with the Agdia Cmm ImmunoStrip.

Results

Infection of inoculated plant species in greenhouse studies.

Symptoms of infection that developed in the plant species inoculated with *C. nebraskensis* were characterized by water-soaked, freckled, necrotic lesions and reddening of the leaf extending from inoculation sites, similar to the symptoms reported and shown on grass species by Langemeier et al. (2014) and Ikley et al. (2015). Seven species consistently developed lesions matching this description: large crabgrass, annual ryegrass, woolly cupgrass, big bluestem, little bluestem, maize, and teosinte (Table 1). Maize and teosinte, used as positive controls, consistently developed large lesions typical of Goss's wilt and thus confirmed favorable conditions for infection and disease development in both replicated studies. In addition, bacterial streaming was consistently observed from inoculated leaves of these seven species, demonstrating that *C. nebraskensis* multiplied and was present at high concentrations inside the leaf tissues (Table 1). Disease severity was also significantly greater in these species, as well as in maize and teosinte, than in the other species in this study (Table 1). Woolly cupgrass, big bluestem, and little bluestem had not been previously reported to be susceptible to infection by *C. nebraskensis*. More leaf reddening was observed in these three species than typically develops on maize. Proso millet and witchgrass developed symptoms and bacterial streaming inconsistently. None of the dicot species developed lesions typical of Goss's wilt or bacterial streaming.

Survival and growth of *C. nebraskensis* on plant species in greenhouse studies. Infected hosts of *C. nebraskensis* supported high populations of the bacterium. Each of the seven species identified as hosts based on symptom development and bacterial streaming were the species that supported populations of $>10^7$ CFU/sample 3 WPI (Table 1, Fig. 1). The nonsymptomatic species, i.e., barley, sunflower, waterhemp, soybean, millet, buckwheat, and pigweed, were the species with populations of *C. nebraskensis* significantly lower

than the population measured on maize ($P = 0.05$). Teosinte had the highest average population, followed by maize, large crabgrass, big bluestem, annual ryegrass, little bluestem, and woolly cupgrass (Fig. 1). The average populations of *C. nebraskensis* measured on proso millet and witchgrass were 10^6 to 10^7 CFU/sample. The colonies selected for further examination were gram positive and tested positive with Agdia Cmm ImmunoStrip, consistent with an identification as *C. nebraskensis*.

All plant species from the Poaceae supported at least 10^6 CFU/sample of *C. nebraskensis* regardless of the presence or absence of symptoms, but dicot species supported lower populations (Fig. 1). For example, barley, spring wheat, oat, and winter ryegrass did not develop symptoms of infection or evidence of bacterial streaming following inoculation with *C. nebraskensis*; however, each supported 10^6 and 10^7 CFU/sample. Among the dicots, sunflower and common waterhemp supported about 10^5 CFU/sample, soybean and buckwheat about 10^3 CFU/sample, and pigweed averaged about 10^4 CFU/sample (Fig. 1).

Infection and survival of *C. nebraskensis* in field studies. *C. nebraskensis* was detected on proso millet, spring wheat, and barley 4 weeks after inoculation. Maize developed typical symptoms of Goss's wilt. *C. nebraskensis* was not detected on the other plant species in the field experiments. Development of other diseases and populations of common epiphytic bacterial species confounded the detection and measurement of populations of this pathogen on all plant species over 4 weeks after inoculation, and some before 4 weeks. Many of the grass species were infected by powdery mildew and rust soon after inoculation with *C. nebraskensis*, making it impossible to monitor infection. The lack of selective media for isolation of *C. nebraskensis* also proved to be a major impediment, as this pathogen grew more slowly in culture compared with many other species of epiphytic bacteria present in the fields.

Discussion

Inoculation experiments were used in this study to identify plant species that can be infected by and support high populations of *C. nebraskensis*. This is the first study to demonstrate the ability of multiple plant species to develop symptoms of infection and to host epiphytic and/or endophytic populations of this pathogen. The results of this work also elucidate different types of interactions between *C. nebraskensis* and plants, provide insights into the ecology of this pathogen, and identify a large potential reservoir of inoculum that may include weeds, crops, and native prairie grasses.

Four categories of interactions between *C. nebraskensis* and plants were identified based on lesion development, bacterial streaming, and pathogen population size on leaves following inoculation studies in a greenhouse environment. One category is primary hosts, which consistently produced expanding lesions and bacterial streaming in addition to supporting high populations of this pathogen. The plant species in this category, all in the Poaceae, were woolly cupgrass, big bluestem, little bluestem, annual ryegrass, large crabgrass, as well as maize and teosinte used as positive check species. A second category is secondary hosts in the Poaceae, which inconsistently produced lesions and bacterial streaming but supported high populations ($>10^7$ CFU/sample) of the pathogen. The species in this group were proso millet and witchgrass, which have not previously been reported to be hosts of *C. nebraskensis*. A third category is sustaining hosts in the Poaceae, which did not develop symptoms or bacterial streaming, but supported epiphytic populations of the pathogen $\geq 10^6$ CFU/sample. Spring wheat, winter ryegrass, oat, and barley were in this group. The final category is dicot nonhosts, which did not develop symptoms or bacterial streaming and supported populations $<10^6$ CFU/sample. The species in this group were common waterhemp, soybean, sunflower, buckwheat, and pigweed.

Woolly cupgrass, big bluestem, and little bluestem were identified as primary hosts of *C. nebraskensis* for the first time in this study. These species developed expanding lesions, bacterial streaming, and supported high pathogen populations. Woolly cupgrass is a weedy grass species that commonly inhabits maize and soybean fields. It may serve as an inoculum reservoir along with other

Table 1. Presence or absence of lesions typical of Goss's leaf blight and wilt, presence of bacterial streaming, and disease reaction^w on inoculated leaves for 18 plant species 3 weeks postinoculation (WPI) in a greenhouse

Plant species	Lesion 3 WPI ^x	Bacterial streaming ^y	Disease reaction ^z
Maize	+	+	5.0 ^a
Teosinte	+	+	5.0 ^a
Large crabgrass	+	+	3.5 ^a
Big bluestem	+	+	4.8 ^a
Annual ryegrass	+	+	3.0 ^a
Woolly cupgrass	+	+	4.5 ^a
Little bluestem	+	+	5.0 ^a
Proso millet	+/-	+/-	2.5 ^{ab}
Witchgrass	+/-	+/-	1.0 ^b
Winter ryegrass	-	-	0.3 ^b
Oat	-	-	0.8 ^b
Barley	-	-	0.3 ^b
Sunflower	-	-	0.0 ^b
Common waterhemp	-	-	0.0 ^b
Soybean	-	-	0.0 ^b
Buckwheat	-	-	0.5 ^b
Pigweed	-	-	0.5 ^b
Spring wheat	-	-	0.3 ^b

^w Results in the table represent two replicated experiments, which were not significantly different.

^x + Denotes lesion development across all experiments and reps.; +/- denotes inconsistent lesion development across experiments and replications; and - denotes that lesions never developed in these studies.

^y + Denotes bacterial streaming 3 WPI was always observed from symptomatic tissue; +/- denotes streaming was inconsistently observed; and - indicates bacterial streaming was not observed.

^z Mean disease reaction on inoculated leaves 3 WPI (0–5 scale, where 0 represents no symptom development) from two replicated experiments. Values followed by the same superscript letter are not significantly different ($P = 0.05$).

common weed species in the midwestern United States, including large crabgrass and several foxtail species, which can be infected by this pathogen (Ikley et al. 2015; Langemeier et al. 2014; Schuster 1975). Controlling these weed species near maize fields may be important for managing Goss's wilt. The native prairie grasses big bluestem and little bluestem are less widespread than many other grass hosts, but development of severe lesions along with their support of high populations of *C. nebraskensis* suggest that they could potentially be important inoculum reservoirs.

In addition to demonstrating that big and little bluestem can be infected by and support high populations of *C. nebraskensis*, this study suggests that these prairie grasses could have a long coevolutionary history with this pathogen and with Goss's wilt in maize. Big and little bluestem were once widespread in native prairies across the central United States and Canadian prairie provinces (Samson and Knopf 1994). Much of the area previously inhabited by big and little bluestem is now consistently planted to maize, and the known distribution of *C. nebraskensis* and Goss's wilt of maize coincides remarkably well with the native range of these two grasses. Potential historical associations between *C. nebraskensis* and big and little bluestem in prairies prior to cultivation of maize have not been studied. This study supports the hypothesis that *C. nebraskensis* may have long existed as a pathogen or an endophyte/epiphyte in these grasses and may have jumped from these hosts to maize. To our knowledge, research has not been done in prairie environments to address the presence or importance of *C. nebraskensis*; however, Zinniel et al. (2002) isolated a *Clavibacter* sp. closely related to *C. nebraskensis* from prairie dropseed, another native grass species in Nebraska. Further research on *C. nebraskensis* and prairie grasses could provide important insights regarding the evolutionary history and ecology of *C. nebraskensis*.

Numerous grassy weeds have been previously reported to develop symptoms of infection following inoculation with *C. nebraskensis* (Ikley et al. 2015; Langemeier et al. 2014; Schuster 1975). Ikley et al. (2015) reported the most recent expansion of the host range of *C. nebraskensis* with their description that the weedy grass species large crabgrass, annual ryegrass, and Johnson grass are hosts based on symptom development and bacterial streaming following inoculation. Two of those species, large crabgrass and annual ryegrass, were

included in the study reported here before their work was published. Our results with these two species support and expand on their results using different genotypes of these grass species. The fact that annual ryegrass is a common cover crop suggests it deserves consideration when managing Goss's wilt, as it may be a commonly planted host in which *C. nebraskensis* can overwinter and subsequently infect maize.

This study is the first to monitor epiphytic populations of *C. nebraskensis* on plant species other than maize and to document that many species can support greater than 10^7 CFU/sample. We used an experimental approach that has been used previously to study the epidemiology and ecology of phytopathogenic bacteria, including species of *Clavibacter*, *Pseudomonas*, and *Xanthomonas* (Barak et al. 2001; Hirano and Upper 1983; Latorre and Jones 1979). Plant-pathogenic species in these genera were reported to reside on weeds and/or grasses near their hosts, and they subsequently could infect the nearby plant host of economic significance.

Proso millet and witchgrass were identified as secondary hosts in the present study. These plants developed inconsistent symptoms and bacterial streaming from symptomatic tissues following inoculation with *C. nebraskensis*. In addition, and potentially of importance, these species could maintain high epiphytic and/or endophytic populations of *C. nebraskensis*. Thus, although the results suggest that *C. nebraskensis* may not be an important pathogen of proso millet and witchgrass, the fact that these species could support high populations of this pathogen suggest they could be important in the ecology and epidemiology of this pathogen, and management of these plant species near maize fields may be important for management of Goss's wilt.

Barley, spring wheat, and oat did not develop symptoms of infection or bacterial streaming after inoculation with *C. nebraskensis*; however, they supported high populations between 10^6 and 10^7 CFU/sample. The absence of bacterial streaming suggests that *C. nebraskensis* may be more important as an epiphyte than an endophyte on these crops. These three crops are widespread and important by acreage in many areas where maize is also produced, and thus may also play a role in the epidemiology of Goss's wilt.

The dicot species included in this study developed neither symptoms of infection nor bacterial streaming after inoculation

Fig. 1. Average epiphytic populations of *Clavibacter nebraskensis* recovered from 18 plant species at 3 weeks postinoculation in two replicated studies in a greenhouse. Barley, sunflower, waterhemp, soybean, millet, buckwheat, and pigweed were the only species with populations significantly different than the population measured on maize ($P = 0.05$). Species with populations $>10^7$ CFU/sample also had consistent symptom development and bacterial streaming, and thus were considered primary hosts of *C. nebraskensis*.

with *C. nebraskensis*, and supported populations less than 10⁶ CFU/sample. However, the fact that *C. nebraskensis* can survive at these levels nearly a month after inoculation, and that soybean, sunflower, common waterhemp, buckwheat, and pigweed commonly grow in areas with maize production, suggests that these dicot species may also play a role in Goss's wilt epidemiology. Although it is not known how long *C. nebraskensis* can grow and survive on these and other dicot species, it may be long enough to allow this pathogen to survive, reproduce, and infect maize.

The field studies demonstrated that *C. nebraskensis* could survive on proso millet, spring wheat, and barley in field environments for at least 4 weeks after inoculation. Other results from the field studies were confounded and inconclusive, primarily due to the prevalence of other diseases in the field studies and the challenges of isolating *C. nebraskensis*. Most of the grass species, including spring wheat, barley, and oat were overtaken by powdery mildew and rust soon after the inoculations at both field locations, making it difficult to monitor infection by *C. nebraskensis*. In addition, this work illustrated the challenges with detecting *C. nebraskensis* on plants from the field. This pathogen is slow growing and difficult to isolate and identify among the myriad of other epiphytic and endophytic bacteria that occurred on the plants in this study. Recently developed PCR-based assays would be useful tools for detecting and quantifying *C. nebraskensis* on field-grown plant samples (Harding et al. 2018; McNally et al. 2016).

This study builds broadly on previous work with *C. nebraskensis* by establishing new host species and demonstrating epiphytic/endophytic survival of this pathogen on multiple plant species in the absence of symptoms of infection. The results provide evidence that the ecology, history, and epidemiology of *C. nebraskensis* and Goss's wilt are likely more complex than previously described. Not only is *C. nebraskensis* a pathogen of potential importance to more grasses, including native prairie species, than previously known; it likely also can survive and grow as an epiphyte and/or endophyte on many different crop and weed species that may serve as inoculum reservoirs for *C. nebraskensis*.

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