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Four Common *Setaria* Species are Alternative Hosts for *Clavibacter michiganensis* subsp. *nebraskensis*, Causal Agent of Goss's Bacterial Wilt and Blight of Corn

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ABSTRACT

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Goss's bacterial wilt and blight, caused by *Clavibacter michiganensis* subsp. *nebraskensis* (Cmn), has reemerged as an important disease of *Zea mays* (corn) in the U.S. Midwest. Results from a 2011 multistate survey indicated that *Setaria* spp. (foxtail) were often present in corn fields with a history of Cmn. The objective of this research was to determine if *Setaria* spp. that are common in the Midwest are susceptible to infection by Cmn. In the greenhouse, seedlings of four *Setaria* spp., including *S. viridis* (green foxtail), *S. faberi* (giant foxtail), *S. verticillata* (bristly foxtail), and *S. pumila* (yellow foxtail), and *Zea mays* (Golden Cross Bantam sweet corn, GCB) were inoculated with a suspension of 1.0×10^7

bacteria cells. The trial was arranged in a randomized complete block design and repeated once. Percent of symptomatic leaf area was visually estimated eight days after inoculation. *S. faberi* exhibited the highest levels of disease among the four *Setaria* spp., with disease incidence similar to what was observed on *Z. mays*. *S. viridis* was the next most susceptible. Symptoms were also observed on *S. viridis*, *S. verticillata*, and were lowest for *S. pumila*. Bacterial streaming was confirmed microscopically and Cmn was reisolated from the four *Setaria* species. Results indicate that these four *Setaria* spp. are susceptible to Cmn, thus serving as potential sources of inoculum.

PRESENCE AND EPIDEMIOLOGY OF GOSS'S WILT

Goss's bacterial wilt and blight (*Clavibacter michiganensis* subsp. *nebraskensis*, Cmn) (18) was first reported in three *Zea mays* L. (corn) fields in Dawson County, Nebraska in 1969 (20). The disease Cmn has since been confirmed in eleven states including Nebraska, Colorado, Kansas, Iowa, Wyoming, Wisconsin, South Dakota, Minnesota, Illinois, Indiana, and Texas (5,7,9,11,12,13). The inoculum Cmn overwinters in infected plant residue from the previous season(s), as well as inside and on seed (13).

Studies on host range were conducted by Schuster (13) to determine what other plant species might serve as alternative hosts to Cmn in the greenhouse. Schuster (13) reported that teosinte (*Euchlaena mexicana* Schrad.), eastern gamagrass (*Tripsacum dactyloides* L.), green foxtail (*Setaria viridis* (L.) Beauv.), grain sorghum (*Sorghum vulgare* L.), sudangrass (*Sorghum bicolor* (L.) Moench ssp. *drummondii* (Nees ex Steud.) de Wet & Harlan), sugarcane (*Saccharum officinarum*), and shattercane (*Sorghum bicolor* (L.) Moench ssp. *arundinaceum* (Desv.) de Wet & Harlan) are susceptible hosts of Cmn. Wysong (19) also reported that barnyard grass (*Echinochloa crus-galli* (L.) Beauv.) was an alternative host, although this species was not confirmed in trials by Schuster (13). Naturally occurring infections have been reported in *Sorghum bicolor* (L.) Moench ssp. *arundinaceum* (Desv.) de Wet & Harlan, *S. viridis* (L.) Beauv., and *Echinochloa crus-galli* (L.) Beauv. (19).

Several *Setaria* spp. are commonly observed growing in row crop fields, along terraces, waterways, field borders, and roadsides. These species include *S. faberi*, *S. viridis*, *S.*

verticillata, and *S. pumila*. As Cmn becomes more prevalent, management of alternative hosts may play an important role in protecting a *Z. mays* crop from this pathogen. *Setaria viridis* has been reported as an alternative host previously and in earlier studies performed by Schuster (13), although *S. pumila* was not a confirmed host for Cmn. Since only *S. viridis* has been reported (13,19) as an alternative host of Cmn, an experiment was designed to confirm *S. viridis* and test three other *Setaria* spp. that commonly occur in Nebraska for their susceptibility to Cmn, using mechanical inoculation.

CONFIRMING SUSCEPTIBILITY

Bacterial isolates and inoculum preparation. More than 600 Cmn isolates were collected from infected, symptomatic corn leaves submitted to the University of Nebraska–Lincoln Plant and Pest Diagnostic Clinic as part of a multistate survey conducted during the 2011 growing season (8). Four isolates were randomly selected with diverse geographic origins in Nebraska and with confirmed virulence following inoculation of Golden Cross Bantam sweet corn (GCB) in the greenhouse that produced typical symptoms (data not shown). The isolates included in the study originated from Dodge, Cheyenne, Hall, and Butler counties in Nebraska.

Inoculum was produced from a mixture of all four isolates. Suspensions were made of each isolate and then mixed to produce the inoculum at a 1:1:1:1 ratio (12). Isolates were grown for four days at 25°C on *Corynebacterium nebraskense* selective medium (CNS) minus LiCl (14), a selective agar medium developed for isolating the Cmn pathogen as described by Gross and Vidaver (4). Bacterial colonies were then suspended in 10 mM PO₄ buffer at a pH of 7.1 (3). Inoculum concentrations were adjusted to a concentration of 1.0×10^7 (13) with a Spectrophotometer (Spectronic 20D+, Thermo Scientific, Waltham, MA), set at 640 nm (10).

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FIGURE 1

Modified multiple-needle inoculation tool, designed to inoculate small, upright narrow leaves (top). Applying pressure to the device releases a bacterial suspension over the wounds created by the inoculation tool (bottom).

Grass seeds. The *S. viridis*, *S. faberi*, *S. verticillata*, and *S. pumila* seeds were provided by the University of Nebraska-Lincoln Department of Agronomy and Horticulture. The seeds were stored in a freezer in the greenhouses at -15°C prior to experimental setup.

Greenhouse conditions and experimental design.

The experimental design was a split-plot arranged in a randomized complete block design, where plants inoculated with bacteria were maintained separate from those inoculated with sterile phosphate buffer to prevent cross-contamination (data not shown). The experiment was repeated in time, and conducted in the University of Nebraska-Lincoln Plant Pathology greenhouse. The greenhouse day/night temperatures were 28/22°C. Supplemental artificial light was used to produce 16 h of light to 8 h of darkness per 24-h period.

Setaria spp. seeds were planted into steam-pasteurized soil in 15.2 cm pots at a seeding rate of 30 seeds per pot, and thinned to five plants per pot one week after emergence. *Setaria* spp. seeds were planted at a depth of 1.5 to 2.5 cm to optimize germination rates. GCB was planted a depth of 3.75 cm. GCB was included in the study as a positive control to insure that the inoculum was virulent and produced typical symptoms (15,17). GCB was planted at five seeds per pot and thinned to three plants per pot. Inoculation took place at 30 days and 24 days after planting for trial 1 and trial 2, respectively. This is when plants were large enough to effectively use the inoculation device (Table 1). Inoculation was conducted via a modified multiple-needle inoculation method as described by Andrus (2). Five replicate pots were inoculated for each species. All five *Setaria* plants and all three GCB in each pot were mechanically inoculated by squeezing the inoculation device (Fig. 1A). Three to five tillers of *Setaria* spp. were inoculated simultaneously at the whorl per squeeze of the inoculation device. The needles on the inoculation device penetrated the leaves through the whorl creating wounds and the subsequent pressure released the bacterial suspension (Fig. 1B).

A modification of Koch's postulates were completed on inoculated leaves that developed symptoms to confirm the identity of the pathogen. Bacteria were isolated from lesions on symptomatic leaf tissue and cultured onto CNS medium after surface sterilization was completed. Agdia ImmunoStrips (ELISA) test kit (Agdia Inc., Elkhart, IN) designed for *C. michiganensis* subsp. *michiganensis* were used to test both symptomatic leaf tissue and cultures. The ImmunoStrips used have proven to be a useful and effective tool in identifying *Cmn* as well as other subspecies of *Cm* (*Clavibacter michiganensis*)(6). The test strips were able to positively identify *Cmn* 100% of the time on samples sent into the University of Nebraska-Lincoln Plant and Pest Diagnostic Center. The test also did not produce any positives when tested on 13 other bacterial pathogens found outside the genus *Clavibacter*. The manufacturer's recommended procedures for testing were used (6). Symptomatic leaf tissue was also examined and confirmed microscopically (20X) for bacterial streaming. Finally, disease symptoms were visually estimated

TABLE 1

Average plant height taken to the center of the whorl at inoculation, days until first symptoms of Goss's leaf blight (freckling) appeared, and percent leaf area affected of four *Setaria* spp. inoculated in the greenhouse with *Clavibacter michiganensis* subsp. *nebraskensis* (10⁷ cfu/ml).

Species (common names)	Average height at inoculation ^x (cm)	Days until symptoms appeared (days) ^y	Percent leaf area affected by disease ^z (%)	
			Inoculated	Non-inoculated
<i>Setaria faberii</i> (giant foxtail)	21,20	4,5	21.5 a	0a
<i>Setaria viridis</i> (green foxtail)	20,19	5,6	12.5 b	0a
<i>Setaria verticillata</i> (bristly foxtail)	18,17	5,5	8.0 c	0a
<i>Setaria pumila</i> (yellow foxtail)	14,14	5,6	4.4 d	0a
<i>Zea mays</i> (Golden Cross Bantam sweet corn)	37,35	5,6	24.0 a	0a

^x Average height measured at the top of the whorl.

^y Trial 1 and 2, respectively.

^z Mean separations were conducted using a Tukey adjustment with trial ($P > 0.0001$) and species ($P > 0.0001$), but with an insignificant interaction ($P = 0.6561$). Letters indicate significant differences within a column at $\alpha = 0.05$.

FIGURE 2

Typical symptoms (freckling and water soaking) of Goss's leaf blight observed on Golden Cross Bantam sweet corn.

FIGURE 3

Bacterial streaming from leaf tissue of Golden Cross Bantam sweet corn observed at 20X magnification.

eight days after inoculation and percentage of leaf area affected by lesion development was estimated visually (0 to 100%) (16).

Statistics. An ANOVA was conducted on both trials with SAS 9.2, (GLM Procedure, Version 9.2, SAS Institute Inc., Cary, NC) using an F-test.

FOXTAIL SPECIES ARE SUSCEPTIBLE TO GOSS'S WILT

Inoculated leaves were observed for development of symptoms. Negative controls showed puncture wounds on the inoculated leaf, but showed no signs of disease development (data not shown). Chlorotic lesions with discontinuous water soaked spots typical for the leaf blight phase of the disease were confirmed on GCB, indicating that the inoculum was viable (Fig. 2). Similar symptoms were also observed and documented on the *Setaria* spp. In both studies, symptoms developed on at least one plant from each of the five replications for all the *Setaria* spp. and GCB. Leaves were tested with the ImmunoStrips and all tested positive (data not shown). Bacterial streaming was also confirmed in all five replications that were inoculated with Cmn (Fig. 3). A modification of Koch's postulates were performed and bacterial colonies typical of (apricot-orange in color, circular, convex, glistening, and butyrous delimited from a darker center zone) Cmn were observed on CNS (18).

Atypical leaves (yellowing or necrotic tissue) in pots inoculated only with phosphate buffer were also tested with the ImmunoStrips, and all were examined microscopically for bacterial streaming, but none of the leaves tested positive with the ImmunoStrips and no bacterial streaming was observed (data not shown).

DISEASE SEVERITY

Symptoms developed on inoculated leaves of *Setaria* spp. in all four species tested. *Setaria* spp. were less susceptible than GCB in trial 1 and trial 2 (Table 1). Percent of leaf area affected by lesion development for *S. faberi* was 22% in trial 1 and 18% in trial 2 (Fig. 4). The next most susceptible species was *S. viridis*. In trials 1 and 2, 12% and 10% of leaf area was affected. *S.*

verticillata had 8% and 5% leaf area affected in trials 1 and 2, respectively. *S. pumila* was the least susceptible in these trials with 5% and <1% leaf area affected for the two trials.

A significant interaction was observed between trial 1 and trial 2 when using a *P* value of 0.05, so trials were not combined for analysis except for disease severity ratings.

FIGURE 4

Symptoms observed on *Setaria faberi*. Similar symptoms observed at 20X magnification on *S. viridis*, *S. verticillata*, and *S. pumila*.

GOSS'S WILT AND THE NEED FOR WEED MANAGEMENT

Plants of the four *Setaria* spp. were found to be susceptible to Cmn, but at varying severity. *S. viridis*, a known alternative host for Cmn, was reported to be susceptible to Cmn in both greenhouse trials, as well as naturally occurring in field settings (13,19). Contrary to previous research by Schuster (13), this research suggests that *S. pumila* is an alternative host for Cmn when similar inoculum concentrations were used. This research also confirmed that both *S. verticillata* and *S. faberi* are hosts of Cmn. This may be the result of a change in the biology of the pathogen over time, genetic differences in the population tested for each *Setaria* spp., or a change in the biology of the host plant.

These possibilities are the reason for using a mixture of isolates. Documented differences in colony color, colony margin development, and bacteriocin production have been reported with Cmn (15,18). More recently, differences in the DNA makeup of Cmn have been reported (1). Research also shows differences in virulence among different isolates collected within the same state (8). If these differences exist, there is the possibility that one isolate may be virulent on *Z. mays*, but not on *Setaria* spp. and vice versa. Therefore, a representative mixture of Cmn was randomly selected from different *Z. mays* growing areas of the state where *Z. mays* and *Setaria* spp. may be growing in the same field.

This research should help producers better manage for Cmn by knowing that control of these four *Setaria* spp. could reduce inoculum. One effective way to reduce inoculum load is to rotate with nonhost crops (13). Since Cmn is a residue-borne pathogen, it is possible for Cmn to over winter in infected corn residue, infect a *Setaria* species growing in a field of soybean (*Glycine max* (L.) Merr.), wheat (*Triticum aestivum*), or any other nonhost crop, and survive in the *Setaria* residues until the following growing season. During subsequent seasons, the Cmn surviving in the *Setaria* spp. residue could infect the corn crop, thus reducing the effectiveness of rotation by not eliminating the inoculum source.

In summary, this research established that *S. pumila*, *S. verticillata*, and *S. faberi* are alternative hosts for Cmn. It also reconfirmed *S. viridis* as an alternative host as reported by Schuster (13), and Wysong (19). Additional research is necessary to test if other species not previously tested could be potential alternative hosts for this particular pathogen.

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