First Report of Goss's Bacterial Leaf Blight and Wilt of Corn Caused by *Clavibacter michiganensis* subsp. *nebraskensis* in North Dakota

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In August of 2011, the North Dakota State University Plant Diagnostic Lab received a hybrid corn (*Zea mays*) leaf sample from Burleigh County in south-central North Dakota (ND). The leaf had long, irregular, water-soaked lesions consistent with Goss's leaf blight of corn. Using a light microscope at 10× magnification, bacterial streaming was observed from the excised edge of leaf tissue. A bacterial suspension was created, streaked onto a semi-selective CNS medium (1), and incubated at 22°C. Dark yellow-orange colonies appeared on the medium after 5 days. Single colonies were subcultured onto additional CNS media. To verify the identity of the bacterial isolate, PCR amplification of the 16S ribosomal DNA from this isolate along with a known *Clavibacter michiganensis* subsp. *nebraskensis* (*Cmn*) isolate collected in Indiana (4) was performed using the eubacterial universal primers 27f and 1525r (3). The 1,431-bp 16S rDNA region was obtained for each isolate and they were compared with each other and with those deposited in NCBI GenBank. Sequence alignment identified only one nucleotide difference between the ND isolate and the Indiana isolate. BLASTn search against the NCBI database showed the first 100 hits were described as *C. michiganensis* or unidentified *Clavibacter* sp. The ND isolate had a two-nucleotide
difference with *Cmn* isolate NCPPB2581 (HE614873), and a three nucleotide difference was found with the *C. michiganensis* spp. *michiganensis* isolate NCPPB 382 (AM711867). To satisfy Koch's postulates, eight corn plants (Golden Cross Bantam) were grown in the greenhouse at 22 to 24°C. Four corn plants were inoculated at growth stage V4-V5 using a suspension of approximately $1 \times 10^9$ CFU/ml from cultures grown on CNS for 6 days. Wounds were created on the fifth leaf approximately 7 cm from the leaf tip using a tongue-seizing forceps outfitted with a rubber stopper composed of pins (2). Simultaneously, 1 ml of the bacterial suspension was delivered into the wounds through a hole on top of the rubber stopper. Four control plants were inoculated with sterile water in a similar fashion. No symptoms were observed on the control plants. After 6 days, long water-soaked symptoms were observed on leaves inoculated with the bacterial suspension. Using leaves with water-soaked lesions, the pathogen was re-isolated onto CNS media and subjected to PCR amplification, and the resulting amplicons were sequenced as before. The sequence of the amplicon from the re-isolation matched that of the original ND isolate. To our knowledge, this is the first account of Goss's leaf blight and wilt identified in ND. As the corn acreage and no-till production systems in the state have increased, the economic implications of this disease may become more significant. Recognition of symptoms and proper identification of this bacterial disease in the field should help reduce unnecessary foliar fungicide sprays.

**References:**