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First Report of *Curvularia inaequalis* and *Bipolaris spicifera* Causing Leaf Blight of Buffalograss in Nebraska

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plant disease

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Disease Notes

First Report of *Curvularia inaequalis* and *Bipolaris spicifera* Causing Leaf Blight of Buffalograss in Nebraska

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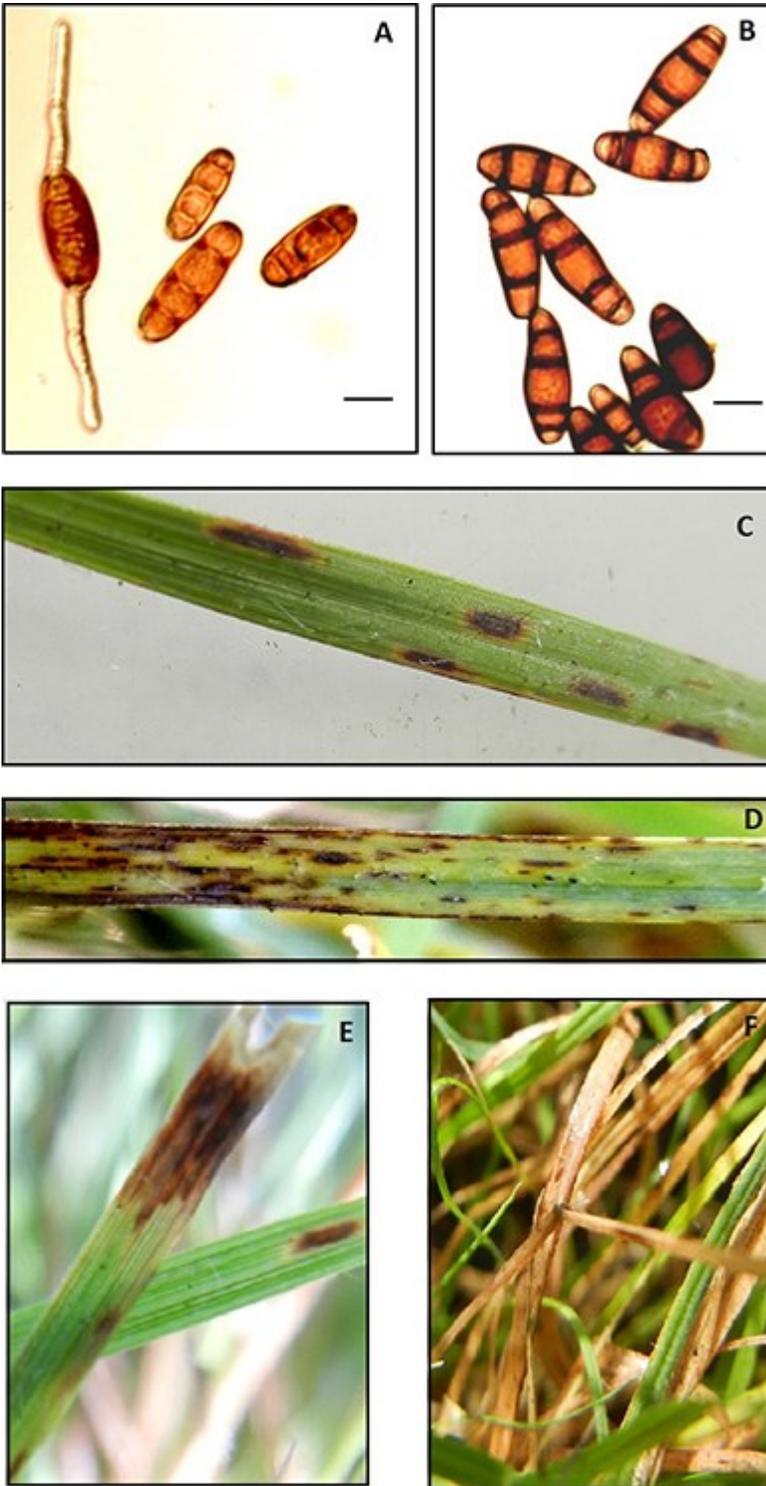
During the summer of 2011, foliar blight was observed on buffalograss (*Buchloë dactyloides* (Nutt.) Engelm.) lawns in Lincoln and Waverly, Nebraska. Disease symptoms were common when buffalograss was growing above 30°C and in drought conditions. Disease symptoms began as dark brown oblong leaf spots, followed by leaf tip dieback and eventual blighting of entire tillers. Leaf infections would progress into patches of thinning turf. Diseased leaf pieces were rinsed in distilled water and placed on 1.5% water agar. Two mitosporic fungal species having conidial morphology of *Curvularia* and *Bipolaris* were isolated. Colonies of *Curvularia* isolates grown on ¼× PDA at 25°C appeared velvety and dark greenish to grayish black after 1 week while *Bipolaris* cultures were brownish gray with olive green margins. The two species were identified as *Curvularia inaequalis* (Shear) Boedijn (1) and *Bipolaris spicifera* (Bainier) Subram. (2). Conidia of *C. inaequalis* were mostly straight to slightly curved, 17.4 to 37.1 × 7.2 to 12.6 ($n = 24$) μm, pale brown to brown, and three to four septate with scarcely protuberant hilum. Conidia of *B. spicifera* were 18.5 to 30.3 × 7 to 11.4 ($n = 20$) μm, ellipsoidal or oblong, light brown, 3-distoseptate with a flattened hilum. DNA confirmation was performed using one isolate of each of the two species. The rDNA-ITS region, amplified with ITS1: 5'-TCCGTAGGTGAACCTGCGG-3' and ITS4: 5'-TCCTCCGCTTATTGATATGC-3', and the *gpd* gene, amplified with *gpd*1: 5'-CAACGGCTTCGGTCGCATTG-3' and *gpd*2: 5'-GCCAAGCAGTTGGTTGTGC-3' (3) were used to confirm taxon of the isolates by comparing

DNA sequences to those in GenBank. Our *B. spicifera* isolate Wv1Bss2 (Accession Nos. KC897667 [ITS] and KC928089 [gpd]) had >99.8% sequence identity to *B. spicifera* strain CCTU 245 (Accessions JX070077 and JX070078) while our *C. inaequalis* isolate Wv3YBss2 (GenBank Accession Nos. KC897663 [ITS] and KC928086 [gpd]) showed >98.6% sequence identity to strain ZM020029 (Accessions HM053665 and HM053653). Pathogenicity of the two species was tested on buffalograss cultivar Prestige. Stolons of Prestige were established in 10 cm square pots filled with Fafard 3B Mix potting medium. The pots of buffalograss were kept in a 30°C greenhouse with a 12-h photoperiod for 12 weeks. One isolate of each species representing each collection site (two isolates per each species) were cultured on ¼× PDA plates and conidial suspensions of 1.5×10^6 spores/ml in sterile water were prepared. Each isolate was inoculated to three pots of Prestige by spraying 15 ml of spore suspension per pot. Control pots of Prestige were sprayed with water. Pots were sealed in transparent plastic bags and every other day, opened for a few hours and plants sprayed with water to encourage infection. Isolates of *C. inaequalis* were more virulent with initial symptoms of foliar spots appearing 7 days after inoculation, followed by leaf tip dieback and necrosis of infected tillers. *B. spicifera* isolates induced similar symptoms 14 days after inoculation. Control pots were asymptomatic. *C. inaequalis* and *B. spicifera* were successfully re-isolated from symptomatic tissue, completing Koch's postulates. To our knowledge, this is the first report of identification of foliar blight causal pathogens on buffalograss in Nebraska.

References:

- (1) A. Sivanesan. Mycol. Pap. 158:1, 1987.
- (2) H. M. Koo et al. Plant Pathol. J. 19:133, 2003.
- (3) M. L. Berbee et al. Mycologia 91:964, 1999.

Supplemental Material



A, Conidia of *Bipolaris spicifera* including a single bipolar-germinating conidium. **B**, Conidia of *Curvularia inaequalis*. **C**, The initial symptoms of greenhouse inoculated buffalograss leaf blight caused by *C. inaequalis*. **D**, Expanding irregular lesions of leaf blight two weeks after inoculation of *C. inaequalis*. **E**, Symptoms of leaf tip dieback on buffalograss caused by *C. inaequalis*. **F**, Dead leaves of buffalograss due to leaf blight. Bar = 10 μm .