Diseases Caused by Fungi and Fungus-Like Organisms

First Report of Leaf Spot Disease on *Microstegium vimineum* Caused by *Bipolaris setariae* in China

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Microstegium vimineum, a Poaceae annual C4 plant that occurs widely south of the Yellow River, China, also causes ecological and environmental damage in the eastern U.S.A. (Stricker et al. 2016). In October 2015, plants with leaf spots were observed by Mingling Road (32.04521°E, 118.84323°N), Nanjing, China. Initially, light brown or brown, round or oval-shaped lesions appeared on the upper surface of leaves. Lesions gradually expanded, and the edges of diseased leaves were lightly curled. Finally, leaves withered or curled and the plant died. Incidence was up to 85% among natural populations. Diseased leaves from the field were surface disinfected (75% ethanol for 30 s; 1% NaOCl for 30 s; 75% ethanol for 30 s; sterile deionized water for 1 min) and placed on water agar (20 g of agar/liter) (Kleczewski et al. 2010). Plates were incubated in the dark at 28°C for 3 days. Leaves, spores, and conidiophores were then examined using light microscopy. Single spores were isolated, plated onto MV agar (30 g of *M. vimineum* leaves, 20 g of agar per liter), and placed under 365-nm black light. Colonies were transferred to PDA; after 4 days colonies measured 83 to 86 mm (diam.) and appeared flat and dark brown, with short, light gray aerial hyphae. Conidiophores were solitary or clustered, light to medium brown, with pale apical color and multiple septa. The upper part was usually geniculate, 5.5 to 9.5 µm wide. Conidia were light yellowish brown to medium yellowish brown, mostly fusiform, straight or curved, fusoid or navicular, often slightly curved, rarely straight, smooth, 5 to 9 (mostly 7) septa, 48 to 70 × 10 to 14.5 µm (av. 57 × 12.5 µm), hilum slightly prominent, and truncated at the base. The fungus was preliminarily identified as *Bipolaris* sp. Four to five *M. vimineum* seeds were planted in pots (10 cm diam.) filled with nutrient soil, placed in a greenhouse, and watered regularly. Four pots

were inoculated with 1×10^5 conidia/ml suspension, at 4 to 5 true leaf stage. Inoculated seedlings were maintained at 80% RH and 28°C for 24 h in the dark and then transferred to a greenhouse. Three pots of uninoculated seedlings were controls. Two days after inoculation, buff-colored, irregular-shaped spots appeared centered on leaf veins. Within a week, diseased leaves became crinkled, and their edges became yellow to brown. By 15 days, large areas of brown spots appeared on the leaves, some leaves turned yellow-brown and severely curled, and 80% of plants died. Symptoms were similar to the field sample. The reisolated fungus was morphologically identical to the original isolate used for inoculation, fulfilling Koch's postulates. DNA was extracted via CTAB from isolates from the field, and the ITS and GPDH gene were amplified using primers ITS1/ITS4 and GPD/GPD2 (Manamgoda et al. 2014), respectively. The ITS sequence (GenBank MW446193) shared 100% identity with *Bipolaris setariae* (MN215638.1), and the GPDH sequence (MW464364) shared 99.83% identity with *B. setariae* (MK144540.1). Field experiments were conducted at the Laboratory Base of Nanjing Agricultural University. Suspensions with concentrations of 10^5 , 10^4 , 10^3 , 10^2 , and 10^1 spores/ml were prepared, distilled water was the control, and there were four replicates of each treatment. Twenty-four plots were randomly arranged; the experimental unit was 50 to 60 plants in 0.5×0.6 m, with ~20 cm between plots. *M. vimineum* plants were inoculated at 3 to 4 true leaf stage. Inoculation was done at sunset, and 60 ml of spore suspension was sprayed onto each plot. Waterproof-breathable black cloth then covered the plots and was removed 36 h later. Temperature was 20 to 28°C. After 10 days, disease index was recorded. SPSS 20 was used for variance analysis, and Origin 9.0 was used to calculate the ED₅₀ and ED₉₀ of the strain MLL-1-5. Symptoms appeared on inoculated *M. vimineum* seedlings immediately after dark treatment. Within a week, all seedlings inoculated with the highest spore concentration were dead. Plants sprayed with water remained healthy. ED₅₀ and ED₉₀ of MLL-1-5 were 1.9×10^1 and 1.4×10^3 spores/ml, respectively, indicating aggressiveness of MLL-1-5 *B. setariae*. After 28 days, infected *M. vimineum* plants did not recover. This is the first report of leaf spot disease on *M. vimineum* caused by *B. setariae* in China. *M. vimineum* is a widely distributed and harmful weed in China and U.S.A. No biocontrol agents against *M. vimineum* are currently available. *B. setariae* may have potential as a biocontrol agent.

References:

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