Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Curvularia intermedia* Causing Leaf Blight Disease on *Microstegium vimineum* in China

Min Tan,¹ Yanxia Fang,¹ Yun Wu,² Richard C. Reardon,² and Sheng Qiang^{1,†}

¹Weed Research Laboratory, College of Life Sciences, Nanjing Agricultural University, Nanjing, China

²USDA Forest Service-Forest Health Assessment & Applied Sciences Team, Morgantown, WV, U.S.A.

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Stiltgrass (*Microstegium vimineum* [Trin.] A. Camus) is an annual C₄ grass of Asian origin whose native range includes India, Pakistan, Nepal, China, Korea, and Japan (Cole et al. 2004). In China, it is mainly distributed south of the Yangtze River, and is an important weed in autumn-maturing dryland crops, orchards, tea gardens, and plantations. With its high shade tolerance, it also invades forest understories and crowds out local vegetation (Warren et al. 2011). From June to August 2019, a leaf disease was observed causing severe defoliation of stiltgrass on the roadside of Sun Yat-sen Mausoleum in Nanjing City, Jiangsu Province, China (32.045964°N, 118.840064°E). Yellow or yellow-brown necrotic spots were observed on leaf tips and margins of the lower canopy, which later expanded to the entire leaf and progressed up the plant. Disease incidence was approximately 75 to 85% in August. Thirty symptomatic leaves were collected, and tissue samples (5 × 5 mm) were surface disinfected with 75% ethanol for 30 s, 0.02% NaClO for 30 s, 75% ethanol for 30 s, and washed twice with sterile water. Tissues were placed on potato dextrose agar (PDA) and incubated at 28°C for 5 days. Twenty-seven morphologically similar isolates were obtained and purified by single-spore culturing for further study. Colonies on PDA were 70 to 85 mm in diameter after 4 to 5 days, initially white becoming gray-green with flocculent aerial mycelia. Conidiophores were solitary or clustered, 85 to 139 × 5 to 8 µm (*n* = 50), and conidia were obclavate to ellipsoid or spindle shaped, brown, and measured 28 to 37 × 13 to 18 µm (*n* = 50) with three false dissepiments. All characteristics were consistent with *Curvularia intermedia* Boedijn (Sivanesan 1987).

The rDNA internal transcribed spacer (ITS), glyceraldehyde-3-phosphate dehydrogenase (GPDH), and translation elongation factor (TEF) of a representative isolate (JSNJ-2019) were amplified using primers ITS1/ITS4, GPD1/GPD2, and EF1-983F/EF1-2218R (Manamgoda et al. 2014). The ITS sequence of JSNJ-2019 (GenBank: MZ613310) showed 99.83% (582/583 bp) identity with *C. intermedia* (GenBank: MF370184 and GU073102); the GPDH sequence (GenBank: MZ701795) showed 99.66% (581/583 bp) identity with *C. intermedia* (GenBank: LT715828) and the TEF sequence (GenBank: OM282974) showed 99.77% (864/866 bp) identity with *C. intermedia* (GenBank: MF370186). Phylogenetic analysis of the TEF sequences using maximum-likelihood and Bayesian methods placed JSNJ-2019 in the same clade with reference strain *C. intermedia* B19. The isolate was deposited in China Centre for Type Culture Collection (CCTCC) (Isolate code: CCTCC AF 2022041). For a pathogenicity assay, 10 healthy *M. vimineum* in plastic pots (five to six leaf stage) were sprayed with 20 ml of conidial suspension (5 × 10⁴ spores/ml); another 10 healthy plants sprayed with sterile water served as controls. All inoculated and control plants were immediately covered with transparent polyethylene bags and kept in a greenhouse at 28 ± 1°C. Bags were removed after 24 h. The assay was repeated three times. Five days postinoculation, inoculated plants showed leaf blight symptoms as observed in the field, but no disease symptoms were observed on controls. Reisolations were performed from inoculated plants, and the reisolated pathogen was confirmed as *C. intermedia* based on morphological and PCR assays (Konstantinova et al. 2002). No pathogens were isolated from controls. Host range tests showed JSNJ-2019 was pathogenic on corn, wheat, sorghum, barnyardgrass, crabgrass, green foxtail, Chinese sprangletop, cynodon, cogongrass, goosegrass, purslane, and bedstraw and nonpathogenic on barley, rice, oat, cotton, bean, peanuts, rapeseed, tobacco, and tea. These findings suggest *C. intermedia* could be used as a biocontrol agent against invasive *M. vimineum* and farmland weeds. However, application of *C. intermedia* as a bioherbicide should be limited to insensitive crop growing areas.

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[†]Indicates the corresponding author.
S. Qiang; wrl@njau.edu.cn