Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Fusarium lateritium* Causing Shoot Dieback of *Acer negundo* in Europe

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Boxelder (*Acer negundo*) is a tree native to North America. In Europe it is considered a dangerous invasive species, and assigned to the highest (fourth) category of environmental hazard (Tokarska-Guzik et al. 2012). The tree can threaten a wide range of ecosystems and compete with the native flora. Shoot dieback was observed on 20% of boxelder in July 2018 and 2019 in Bryzgiel (N53°59.963' E23°04.324') in northeast Poland. Young trees (10 to 15 years old) with visible symptoms were observed in a small group on a rural roadside. Infected shoots were chlorotic. There were visible shallow cracks on the bark and brown discoloration in sapwood inside infected branches. Symptomatic shoots were collected in sterile envelopes and surface disinfected with 95% ethanol. Twelve wood fragments were cut from the border of living and dead tissue, and then divided into 3 to 5 mm pieces, placed on potato dextrose agar (PDA) medium, and incubated at 21°C. After 10 days 10 *Fusarium* spp. strains were obtained. Pure cultures were derived by monospore isolation. The identification of the isolates was initially based on morphology and molecular genotyping (Leslie and Summerell 2006). On PDA, strains produced white, dense, floccose aerial mycelium with a pink surface. The underside of the Petri dish was brown. Growth of the colony was relatively slow and reached 3.5 cm in diameter after 2 weeks. Microscopic observation revealed the presence of

macroconidia in a few orange sporodochia. Macroconidia were slightly curved, with dimensions of 38 to 45 × 3.2 to 3.5 µm, 4 to 5 septate, with well-formed foot cells and a beak on the apex. On aerial hyphae, single intercalary chlamydospores were present. Microconidia were not found. Morphological identification was confirmed by sequencing the ITS regions and the TEF-1α and β-tubulin genes for representative isolates. Mycelia were grown on potato dextrose broth and freeze dried prior to genomic DNA extraction using the CTAB method. Sequences of two isolates were deposited in GenBank as MN186748 and MN588156 for ITS; MZ191070 and MZ191072 for TEF-1α; and MZ191069 and MZ191071 for TEF-1β. BLASTn search in the NCBI database revealed 100, 98, and >99% similarities of ITS, TEF-1α, and β-tubulin with *F. lateritium* isolates LC171689, KT350607, and FN554618, respectively. A pathogenicity test was conducted on five first year 0.6 to 0.8 cm diameter shoots from a 10-year-old tree. Before inoculation their surface was disinfected with 95% ethanol. Then, bark on the twigs was split longitudinally with a sterile blade and pieces of 10-day-old aerial mycelium grown on PDA were applied on the wound sites. Control samples were inoculated with sterile distilled water only. Inoculated areas were covered with parafilm. The first sign of infection was observed after 3 weeks as a dark lesion in the place of inoculation and chlorosis. Three weeks later a brown ring on the sapwood was seen in the shoot cross-section. Morphologically identical to the original, *F. lateritium* isolate was reisolated from the infected tissues, thus fulfilling Koch's postulates. *F. lateritium* is a species closely associated with trees and shrubs (Leslie and Summerell 2006). However, it has not been recorded on boxelder and this is the first report of *F. lateritium* causing dieback of boxelder. According to the Enemy Release Hypothesis (Elton 1958), new pathogens appearing on alien species can be an indicator of developing environmental resistance to the alien species, which indicate the grade of their domestication. This kind of notification poses a crucial role in invasion monitoring and the search for new biocontrol methods for invasive plant species.

References:

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