Disease Notes

Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Rhizoctonia solani* AG 4 Causing Root and Stem Rot of Stevia in Delaware and Maryland

A. C. Kessler and A. M. Koehler[†]

Department of Plant and Soil Sciences, University of Delaware, Newark, DE 19716

Plant Dis. 104:3076, 2020; published online as <https://doi.org/10.1094/PDIS-01-20-0214-PDN>. Accepted for publication 19 May 2020.

Stevia (*Stevia rebaudiana* [Bertoni] Bertoni) is an emerging crop in the United States used as a nonnutritive sweetener. Stevia seedlings are established in greenhouses by propagative cuttings or from seeds. Seedlings are then transferred to the field approximately 12 to 15 weeks after sowing. Symptomatic seedlings were observed in newly transplanted field sites in Georgetown, Delaware, and Salisbury, Maryland, throughout May and June 2019. Symptoms included wilting, stem lesions, shredded stem tissue, and root necrosis that resulted in the death of approximately 3 to 5% of plants in each field. At the onset of disease, five symptomatic plants were collected at each site, and 3-cm pieces of root and stem tissue were surface disinfested and plated onto potato dextrose agar, amended with 50 µg/ml of penicillin G and streptomycin sulfate. Isolates were incubated at 23°C for 7 days and observed for colony morphology. Tan colonies had mycelia with right-angle branching, septations near the branch, and three to eight nuclei per cell, consistent with the description of *Rhizoctonia solani* Kuhn (Sneh et al. 1991). After extracting DNA, pathogen identification was confirmed for a representative isolate from each state by sequencing of the internal transcribed spacer (ITS) region of nuclear ribosomal DNA using primers ITS4/ITS5 (White et al. 1990). All isolates were identified as *R. solani* AG 4 (99.5% sequence identity with GenBank accession no. MN106332.1). Sequences were deposited in GenBank as accession numbers MT009023 to MT009024 and MT009025 to MT009026, for Delaware and Maryland isolates, respectively. Koch's postulates were

confirmed twice for each of the isolates by inoculating 15-week-old greenhouse-grown stevia plants in 8-cm-diameter pots in sterile soilless media. Colonized oat grains served as the inoculum. Oats were sterilized on three consecutive days and then inoculated with colonized agar plugs of the Delaware or Maryland isolate. Oats were incubated at room temperature for 5 days to allow fungi to thoroughly colonize the oats. Three colonized oat grains were buried at a depth of 5 mm, 2 cm from the base of each plant, with four replicate plants for each isolate. Two noninoculated oat grains were buried in each of the four plants that served as the control. Plants were maintained at 26 ± 2°C, watered once daily with a 12-h/12-h light cycle, and observed over a 5-week period. Within 2 weeks, plants inoculated with the Delaware isolate exhibited symptoms of wilting, stem lesions, stem shredding, root necrosis, and plant death. Plants inoculated with the Maryland isolate developed symptoms at 4 weeks, and control plants did not develop symptoms. Stem and root pieces were collected and surface disinfested, and *R. solani* AG 4 was reisolated from all inoculated plants and confirmed by culture morphology and sequencing (99.5% sequence identity with GenBank accession no. MN106332.1). To our knowledge, this is the first report of *R. solani* AG 4 causing root and stem rot of stevia in North America. Hilal and Baiuomy (2000) reported root rot of stevia by *R. solani* in Egypt. Plant establishment is the most expensive component of U.S. production, so it is important to monitor pathogens with potential to kill transplanted stevia and reduce overall plant stands. Current practices aim for perennial production of stevia in the southeast United States, and diseases that reduce establishment affect yield not only in the first year but also in all subsequent years of production.

References:

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The author(s) declare no conflict of interest.

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Keywords: fungi, field crops, etiology

[†]Indicates the corresponding author.
E-mail: akoehler@udel.edu (A.M.K.)