Diseases Caused by Fungi and Fungus-Like Organisms

Taxonomical Identification of *Clarireedia* Species Causing Dollar Spot Disease of Turfgrass in Georgia

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Dollar spot caused by *Clarireedia* spp. is an important disease of all warm and cool season grasses in the USA, including Georgia. Symptoms (i.e., white to straw-colored circular lesions on leaf blades and large irregular sunken patches on turf swards) were regularly observed with ~50% incidence from early March to late May as well as September to November in the turfgrass fields at University of Georgia–Griffin Campus (lat., 33.26; long., -87.27). Symptomatic plant samples were collected in September 2019 from bermudagrass (BG) (*Cynodon dactylon*), creeping bentgrass (CB) (*Agrostis palustris* syn. *A. stolonifera*), zoysiagrass (ZG) (*Zoysia japonica*), and seashore paspalum (SP) (*Paspalum vaginatum*). Center portions of diseased leaves were cut into 1- to 2-cm pieces, surface disinfected (10% bleach for 2 min, 80% ethanol for 2 min, and rinsed with sterile water three times), and dried in a sterile hood for ~5 min. Disinfected leaf segments were plated on PDA and incubated at room temperature (RT). White fluffy mycelium was visible for all isolates after 24 h of incubation. DNA was isolated from all four isolates using the CTAB method (Doyle and Doyle 1987), and the rDNA ITS region was amplified using ITS4/ITS5 primers (White et al. 1990). Based on sequence comparison in the NCBI database, the isolate from CB was identified as *Clarireedia jacksonii* with 100% sequence homology to MF964320, whereas the isolates from BG, ZG, and SP were identified as *Clarireedia monteithiana* with 99.60, 100, and 99.60% sequence homology to KF545806, respectively. The ITS sequences of the isolates collected from CB, BG, ZG, and SP were deposited in GenBank: MT497874, MT497855, MT497856, and MT497854, respectively. Furthermore, based on sequence alignment with ClustalW, the isolate from CB had the combination of all 28

single nucleotide polymorphisms (SNPs) specific to *C. jacksonii*, and isolates collected from BG, ZG, and SP all had the SNP combination specific to *C. monteithiana* (Salgado-Salazar et al. 2018). Our results support the host specialization described by Salgado-Salazar et al. (2018) in natural infections: *C. jacksonii* was identified on cool-season turfgrass, whereas *C. monteithiana* was uniquely found on warm-season species. Pathogenicity tests were conducted on BG ‘Princess’, CB ‘Penn A1/A4’, ZG ‘Zorro’, and SP ‘Seastar’ with three replications for each treatment. Plant materials were prepared in Kord Presto sheet pots (10 × 10 cm) filled with Sun Gro professional growing mix. A single mycelial plug from a pure culture of the pathogens was transferred into a sterile 125-ml Erlenmeyer flask containing 100 ml of potato dextrose broth and shaken at 150 rpm at RT. The 7-day-old mycelium suspension of each isolate was used to inoculate all four turfgrass species using a research track sprayer (Devries Manufacturing), and then the inoculated plants were placed in a dew chamber with high humidity for 48 h. Enough spacing between the treatments was provided inside the dew chamber to prevent accidental contamination. Controls plants were inoculated with potato dextrose broth using a research track sprayer. Plants were transferred to a greenhouse bench. Typical symptoms of dollar spot (white/tan lesions and sunken patches) were visible 5 days after inoculation; the control plants had no disease development. All isolates of the dollar spot pathogen used were able to cross-infect all four turfgrass species. *C. jacksonii* and *C. monteithiana* were reisolated from the artificially inoculated plants. This is the first report on the taxonomical identification of *C. jacksonii* and *C. monteithiana* pathogens causing dollar spot on turfgrass in Georgia, the first report on taxonomical identification of *C. monteithiana* on ZG in the USA, and the first report of cross-infection potential of *Clarireedia* species in the greenhouse under artificial inoculation. This information will be useful in effective identification and management of dollar spot disease of turfgrass.

References:

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