First Report of Sclerotium stem rot caused by Athelium rolfsii on Stevia rebaudiana in southwestern France

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Stevia rebaudiana is a perennial species that accumulates steviol glycosides in its leaves. These natural compounds produce an intense sweet taste but are non-caloric and are hence in demand by consumers to reduce daily sugar consumption. The cultivation of S. rebaudiana has recently become popular in France. In August 2017, disease symptoms similar to a sudden wilting were observed in a S. rebaudiana 0.5 ha commercial field in Sainte-Livrade (N44° 23' 48''; E0°35'13''), France. Symptoms first appeared on the aerial parts, resulting in wilting and drying of the entire plant. Additional symptoms included lesions on the stem at the collar base with abundant white mycelium and round-white to dark-brown small globoid sclerotia were observed on diseased plants. These symptoms were also observed on nearby pepper crops. The outbreaks of infection were increasing with time. Disease incidence was estimated at approximately 17%. To isolate the pathogen, the collar of one diseased plant was collected and incubated in moist chamber for 2 days at 24°C. Mycelia was removed from the diseased plant parts and placed on MAC (malt extract agar amended with 50 mg/liter chloramphenicol) and incubated at 22°C in a growth chamber with a 12 h photoperiod. After 7 days, immature sclerotia were abundantly formed, and after ten days small globoid, white sclerotia were approximately 1 to 3 mm diam. and became dark brown with age. The freshly isolated pathogenic fungus was examined microscopically. The white mycelia had clamp connection typical of that described for A. rolfsii (Mordue 1974). No sexual reproductive structures were observed. Genomic DNA of one isolate (17SCL_STEV1) was extracted as described (Hastoy et al. 2019). The Large ribosomal subunit locus (LSU rRNA) was amplified and sequenced with primers LROR / LR5 (Stielow et al. 2015). The length of the amplicon was 884 bp (GenBank accession MK680087). Phylogenetic analysis was done with the neighbor-joining method in MEGA7. Isolate 17SCL_STEV1 was identified as A. rolfsii and presented 99% sequence similarity with A. rolfsii isolate DGADY14 from Capsicum annuum. To conduct pathogenicity testing, mycelium was cultivated on MA (Malt-Agar) medium incubated at 22°C for 6 days in the growth chamber. Colonized MA plugs (0.5 diam.) were removed and four were applied to the stems of 5 two-month-old S. rebaudiana cv. Shoutian III plants following injury by a scalpel. For the control, stems were wounded and inoculated with sterile MA plugs with the same method. Plants were kept in a growth chamber at 22°C with a 12-h photoperiod and 85% humidity. After 2 days, lesions appeared on the collar. White mycelium and incipient sclerotia were observed on the lesion and soil surface and turned into typical brownish sclerotia. Inoculated plants wilted and died after 5 days. Three re-isolation were performed once by sampling mycelium from symptomatic plants. Isolates were confirmed as A. rolfsii based on morphological and molecular characteristics. Although there are previous reports of A. rolfsii on S. rebaudiana in India (Kamalakannan et al. 2007), in the USA (Koehler and Shew 2014), Italy (Carrieri et al. 2015) and Greece (Chatzivassiliou et al. 2015), to the best of our knowledge, this is the first report of A. rolfsii causing Sclerotium stem rot of S. rebaudiana in France. This is of primary importance given the damage to yield and the difficulty of controlling A. rolfsii (Punja 1985).

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Figure S1. Sclerotium rot on Stevia rebaudiana and mycological characteristics of the pathogenic fungus, Athelia rolfsii. A and B typical symptoms of plant wilting and drying in the field; C white mycelium formed on the collar of infected plant from the field after 1 day of incubation in a moist chamber; D colony of A. rolfsii with mycelial mat and sclerotia grown on MAC medium after 12 days of incubation; E clamp connections structure formed on the fungus hyphae (arrow); F symptoms (wilt) and signs (white mycelium on the stem and near the soil line) appeared 2 days after artificial inoculation (left; control on the right).

Figure S2. Phylogenetic tree of isolate 17SCL_STEV1 and related sequences from pepper and from NCBI were constructed by MEGA7. Branch stability was estimated by bootstrapping with 1000 replicates, support values are shown at the branch points. The analysis involved 16 nucleotide sequences. All ambiguous positions were removed for each sequence pair. There were a total of 446 positions in the final dataset.