Diseases Caused by Fungi and Fungus-Like Organisms

First Report of *Stemphylium eturmiunum* Causing Postharvest Rot on Tomato (*Solanum lycopersicum*) in Italy

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Italy is the largest tomato (*Solanum lycopersicum*)-producing country in Europe with a cultivated area of 97,092 ha and a production of 5,798,103 t/year in 2018 (FAOSTAT 2020). During July 2020, a postharvest rot occurred in fresh 'Piccadilly' tomatoes cultivated in Sicily (Pachino, RG) and commercialized in northern Italy (Torino, TO). Affected fruit showed circular black rot on the blossom end. The rot had an average incidence of 7% of the fruits, in three batches of 100 tomatoes each. Isolation was carried out by cutting pieces of symptomatic rotten fruits. The fragments were surface disinfected with 1% sodium hypochlorite for 30 s, rinsed in sterile water, and air dried. Five fragments were cut and plated onto potato dextrose agar (PDA) supplemented with streptomycin and were incubated at 24 ± 1°C in the dark for 5 days. Representative colonies were transferred onto potato carrot agar (PCA), and morphological observations were performed as described by Woudenberg et al. (2017) after 7 and 14 days. Colonies were olive-green, flat with regular margins, and conidia were mid to deep brown, solitary, ovoid or ellipsoid (17.39 ± 2.04 × 10.59 ± 3.30 µm), with transverse and longitudinal septa. Based on morphological observations the isolates were identified as *Stemphylium eturmiunum* (Simmons 2001). Species identification was confirmed by sequencing rDNA internal transcribed spacer (ITS) using primers ITS1/ITS4 (White et al. 1990), *cmdA* gene region using primers CALDF1/CALDR2 (Lawrence et al. 2013), and *gapdh* gene region with primers gpd1/gpd2 (Berbee et al. 1999). Six amplified sequences per region (accession nos. MW158387 to MW158398 and MW159746 to MW159751) were BLAST searched in GenBank, obtaining >99% identity with ex-type strain of *S. eturmiunum* strain CBS 109845 (accession no. KU850541) for ITS and 100% identity (accession nos. KU850831 and KU850689) for *cmdA* and *gapdh*, respectively. To confirm the species, DNA sequences were aligned with CLUSTAL W with closely related species of *Stemphylium* reported in the last revision of the genus (Woudenberg et al. 2017), and a phylogenetic analysis with the neighbor joining method based on the Tamura-Nei model + gamma distribution (bootstrap 1,000) was performed. The phylogenetic tree confirmed the identity of the isolates as *S. eturmiunum*. To fulfill Koch's postulates, pathogenicity tests were conducted on *S. lycopersicum* cv. Piccadilly fruits. Tomatoes were surface sterilized with 1% sodium hypochlorite and air dried. Fruits (five fruits per isolate) were wounded (two injuries of 3 mm each) and inoculated with a spore suspension of 1×10^5 cell/ml obtained from 15-day-old PCA cultures, as in Spadoni et al. (2020). Negative controls were wounded and inoculated with sterile deionized water. Symptoms occurred on all fruits inoculated after 12 days at 24 ± 1°C, and *S. eturmiunum* was reisolated from inoculated fruits on PCA; the control remained symptomless. Reisolated colonies were molecularly identified as *S. eturmiunum*. In Italy a different species, *S. vesicarium*, was reported on tomato (Porta-Puglia 1981), and *S. eturmiunum* was described as a postharvest pathogen of tomato in China, Greece, New Zealand, and the United States (Vaghefi et al. 2020; Woudenberg et al. 2017), and from fruits commercialized in the Danish and Spanish markets (Andersen and Frisvad 2004). To the best of our knowledge, this is the first report of *S. eturmiunum* causing postharvest rot on tomato in Italy. The occurrence of this pathogen further stresses the importance of careful handling to prevent fruit cracking and of preharvest control strategies.

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