

First Report of Shoot Blight, Canker, and Gummosis Caused by *Neoscytalidium dimidiatum* on Citrus in Italy

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In September 2008, a new disease was noticed in eastern Sicily, Italy in a 2-year-old regrafted citrus orchard with approximately 1,500 plants of sweet orange (*Citrus sinensis* (L.) Osbeck cv. Tarocco Scirè) on sour orange rootstock. Symptoms on the sweet orange scion consisted of blight of vigorously growing shoots and a sooty canker on shoots and rootstock trunks, resulting in shoot dieback to the cankered area. Masses of black fungal spores appeared under the bark and on the canker surface. Abundant gummosis was frequently associated with the affected tissues. Of the 1,500 plants surveyed, 12% were infected. A *Scytalidium*-like fungus was isolated consistently from symptomatic tissues on 2% potato dextrose agar (PDA). Conidia were ellipsoid to ovoid, hyaline, with an acutely rounded apex, truncate base, initially aseptate, becoming brown and two-septate at maturity, (10-) 12 to 13 (-14) × (4-) 5 (-6) µm. Mycelium was branched with septate, brown hyphae that disarticulated into 0- to 1-septate phragmospores (toruloid state). Genomic DNA was extracted from mycelia of single-conidial isolates cultivated on malt extract agar. Primers V9G and ITS4 were used to amplify the nuclear rRNA operon spanning the 3' end of 18S rRNA gene, the internal transcribed spacers, the 5.8S rRNA gene, and a part of the 5' end of the 28S rRNA gene (1,2). Both PCR primers were used to sequence directly the entire amplicon. DNA sequences of two isolates (CBS 124887 and 124888) were deposited in GenBank (Accession Nos. GQ330902 and GQ330903, respectively). These sequences were 100% identical in more than 545 nt to GenBank Accession Nos. AY213688 and FJ648577. On the basis of morphological characters and molecular data, the fungal isolates were identified as *Neoscytalidium dimidiatum* (Penz.) Crous & Slippers (2,3). Pathogenicity tests were conducted on five 2-year-old potted plants of sweet orange cv. Tarocco Scirè and lemon cv. Femminello Zagara Bianca, both grafted on sour orange. Eight 5-cm mycelial plugs of a single-conidial isolate were placed in wounds made with a sterile blade in the inner bark of plant stems and branches. Inoculation wounds were wrapped with Parafilm. The same number of plants inoculated with sterile PDA plugs served as controls. Inoculated plants were maintained in a growth chamber at 25 ± 1°C and 90 to 95% relative humidity. After 2 weeks, all inoculated plants developed gummosis originating from the inoculation point. Shoot blight and death of the entire plant were observed within 6 months on all inoculated plants. No differences were observed among the two citrus species. Control plants remained healthy. *N. dimidiatum* was reisolated from the infected plants and identified as described. To our knowledge, this is the first record of a disease caused by *N. dimidiatum* on citrus in Italy. The pathogen has been previously observed to infect freeze-damaged limbs of citrus in California, inducing a disease named Hendersonula branch wilt (4). This pathogen on citrus is important mainly as a wound-invading pathogen, therefore posing a serious threat to regrafted citrus orchards.

References: (1) R. Cheewangkoon et al. *Persoonia* 21:77, 2008. (2) P. W. Crous et al. *Stud. Mycol.* 55:235, 2006. (3) E. Punithalingam and J. M. Waterston. No 274 in: *Descriptions of Pathogenic Fungi and Bacteria*. CMI, Kew, Surrey, UK, 1970. (4) J. O. Whiteside. Page 29 in: *Compendium of Citrus Diseases*. The American Phytopathological Society, St. Paul, MN, 1988.



Fig. 1. Shoot blight and trunk necrotic lesion on regrafted sweet orange cv. Tarocco Scirè on sour orange rootstock caused by *Neoscytalidium dimidiatum* (courtesy G. Polizzi).



Fig. 2. Large necrotic areas and gummosis on sour orange rootstock and shoots of sweet orange cv. Tarocco Scirè caused by *Neoscytalidium dimidiatum* (courtesy G. Polizzi).

