



First report of clementine trunk and branch canker caused by *Phytophthora citrophthora* in Tunisia

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Citrus is an important crop in Tunisia, both economically and socially. Clementine production has increased in recent years in Tunisia after the introduction of new varieties such as 'Marisol' and 'Hernandina' via the FAO's program in 1996 from Corsica, France. A few years after planting, a new disease was observed in the Cap Bon peninsula in northeastern Tunisia on clementine (cv. Hernandina), characterised by trunk and branch cankers leading to death of the trees (Fig. 1).

Samples were collected from bark near the canker lesion. The samples were washed, surface-sterilised by immersing in ethanol for 5 to 10 seconds, and dried on filter paper. Each sample was cut in pieces 2–4 mm wide and placed in PARP selective agar containing cornmeal agar with 10 µg/l pimaricin, 200 µg/l ampicillin, 10 µg/l rifampicin and 20 µg/l pentachloronitrobenzene. Plates were incubated at 24°C in the dark and examined within two to three days. Pure cultures of a *Phytophthora* sp. were obtained by transferring hyphal tips onto potato dextrose agar. Colony pattern descriptions were noted after seven days' growth (Fig. 2). Cultures were transferred to V8 juice agar to determine morphological descriptions (Fig. 3). Six isolates (E1P1, E1P3, E1P2, E4P4, E4P3 and E5P3) were characterised by amplification and sequencing using the universal primers ITS1/4 (White *et al.*, 1990). The sequences of these isolates were deposited in GenBank (Accession Nos. KX269827–KX269832 and have a high identity (97–99%) with *P. citrophthora* strains CNRcitro33RH (KT148903) and GL-Pci-4 (GU133068). Morphological and molecular studies confirmed that the causal agent causing citrus death in orchards was *P. citrophthora*.

Pathogenicity tests were done in a greenhouse on two-year-old clementine (cv. Hernandina) grafted on Carrizo citrange rootstock. A V8 juice agar plug containing sporangia from isolate E1P1 was placed on a wound in direct contact with the cambium. The wound was wrapped with foil, moistened and sealed with a strip of paraffin film to prevent drying. Six plants were inoculated with *P. citrophthora* and two additional plants inoculated with sterile V8 juice agar discs as controls. The first symptoms began to appear one week after inoculation, including gum exudation and onset of canker expansion. Two weeks later, the scion began to decline, and after three weeks, the scion died, persisting only at the rootstock. Non-inoculated controls remained intact. *Phytophthora citrophthora* was re-isolated successfully and Koch's postulates were fulfilled (Fig. 4).

Phytophthora citrophthora has been reported to cause brown rot of citrus in Tunisia (Fawcett, 1930). However, to the authors' knowledge, this is the first report of trunk and branch canker caused by *P. citrophthora* in Tunisia on clementine. This new syndrome was reported first in Spain in 2008 (Alvarez *et al.*, 2008) and then in South Africa in 2010 (Schutte *et al.*, 2010). This disease could have a high economic impact as clementine production increases in Tunisia. The outbreak of this new disease in Tunisian orchards might be explained by an increased planting of the susceptible cv. Hernandina and the appearance of a new virulent strain of the pathogen, belonging to the A2 mating type (Cohen *et al.*, 2003).

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Figure 1

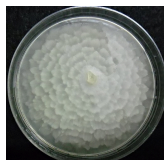


Figure 2

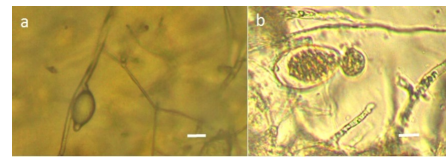


Figure 3



Figure 4

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