First Report of *Phytophthora pistaciae* Causing Root and Collar Rot on Nursery Plants of *Pistacia lentiscus* in Italy

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In April 2019, a survey carried out in a public forest nursery in Sardinia (Italy) (39°57'39"N; 8°36'02"E) revealed the presence of typical *Phytophthora* symptoms such as leaf reddening, wilted shoots and dieback associated with root and collar rot on 3-year-old potted seedlings of lentisk (*Pistacia lentiscus* L.). Approximately 60% of 240 potted plants, obtained from seeds collected in natural areas in Sardinia, were symptomatic. To isolate *Phytophthora*, rhizosphere soil and root samples of seven symptomatic seedlings were baited with *Quercus suber* and *Sambucus nigra* leaves as described by Linaldeddu et al. (2014). After 5 days, leaves showing dark spots were plated on potato dextrose agar (PDA) supplemented with 100 ml/L of carrot juice, 0.013 g/L of pimaricin and 0.05 g/L of hymexazol. *Phytophthora* colonies characterized by an aerial and compact mycelium without a distinct pattern were isolated from all samples. Sporangia produced on PDA plugs immersed in unsterile pond water, were nonpapillate, persistent, ellipsoid, rarely obpyriform (41.6 to 100.4) × (25.1 to 59.3) µm, with a length/width ratio of 1.76, proliferating internally and externally. Hyphal swellings were globose to irregular, while no chlamydospores were observed. All isolates produced smooth-walled oogonia (25.1 to 39.5 µm) with chiefly amphigynous
antheridia after 2 weeks on carrot agar (CA) at 20°C in the dark. Morphological features were in close agreement with those reported for *P. pistaciae* Mirabolfathy (Mirabolfathy et al. 2001). The identity of all isolates was confirmed by sequencing the internal transcribed spacer region (ITS) using the primers ITS1 and ITS4. DNA extraction, PCR amplification reactions and DNA sequencing were carried out according to Linaldeddu et al. (2016). BLAST searches in GenBank showed 100% identity with reference sequences of *P. pistaciae*, including the ex-type culture P19883 (FJ746648). Two representative isolates of *P. pistaciae* (AM43 and AM53) were stored at 10°C under water at the Culture Collection of the TeSAF Dept, University of Padova, and the sequences deposited in GenBank (MN656156 and MN656157). The pathogenicity of both *P. pistaciae* isolates was evaluated by inoculating fourteen 3-year-old lentisk seedlings per isolate. After disinfecting the bark with 90% ethanol and removing a piece of outer bark (5 mm) with a sterile cork borer, the seedlings were inoculated with a same sized agar-mycelium plug cut from the margin of a 5-day-old colony. Seven control plants were inoculated with a sterile CA plug. All plants were kept in a greenhouse at 20 to 26 °C in natural daylight. After three weeks, all plants inoculated with *P. pistaciae* showed wilted shoots and inner bark necrotic lesions spreading up and down from the inoculation site. The average lesion size was 5.9 ± 3.1 cm for the isolate (AM43) and 4.9 ± 1.4 cm for the isolate (AM53). *Phytophthora pistaciae* was successfully reisolated from all the inoculated plants, fulfilling Koch’s postulates. On control seedlings, only a small brown discoloration restricted to the inoculation point was observed. *Phytophthora pistaciae* is reported as one of the main pathogens involved in the aetiology of pistachio (*Pistacia vera* L.) gummosis in Iran (Mirsoleimani and Mostowfizadeh-Ghalamfarsa 2013). This is the first report of *P. pistaciae* outside of Iran and as a lentisk pathogen. Considering the high virulence of this exotic pathogen, this discovery could pose a serious threat to lentisk ecosystems and pistachio cultivation in the Mediterranean area.

References:


Figure 1. Disease symptoms caused by *Phytophthora pistaciae* on lentisk seedlings.

Figure 2. Colony morphology of *Phytophthora pistaciae* after 7 days at 20°C in the dark on potato-dextrose-agar (a), malt extract agar (b) and carrot agar (c). Mature non-papillate, ellipsoid and persistent sporangium (d), sporangium releasing zoospores (e), empty sporangium with nested and extended proliferation (f), external proliferation (g), oogonia with amphigynous antheridia (h). Symptoms observed on lentisk seedlings three weeks after inoculation with *P. pistaciae* (i). Control seedling (j). Scale bars = 50 µm.
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