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Alternaria alternata causes leaf spot and fruit rot on loquat (*Eriobotrya japonica*) in Greece

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Abstract In 2011, a leaf spot and fruit rot disease was observed on loquat trees (*Eriobotrya japonica*) in Thessaloniki, Greece. The pathogen was identified as *Alternaria alternata* (Fr.) Keissl. on the basis of morphology and ITS sequencing. A pathogenicity test was performed and Koch's postulates were confirmed by re-isolation of the fungus *A. alternata* from artificially inoculated leaves and fruits. This is the first report of *A. alternata* as the cause of leaf spots and fruit rot on loquat in Greece.

Keywords *Eriobotrya japonica* · *Alternaria alternata* · ITS sequencing · Pathogenicity

The loquat (*Eriobotrya japonica*) is a subtropical tree of the rose family (Rosaceae) grown commercially for its edible fruits. However, the evergreen loquat is frequently planted in parks and gardens as an ornamental plant.

A leaf spot disease of loquat (*Eriobotrya japonica*) was observed during 2011 in the urban area of Thessaloniki (Northern Greece, Central Macedonia) on trees planted for landscaping. The symptoms recorded were small, 2 to 6 mm in diameter, circular, brown necrotic spots over the foliage. Each spot consisted of a green–yellow halo surrounding a necrotic lesion (Fig. 1). The spots gradually enlarged in size and later became irregular in shape or remained circular with concentric rings or zones (Fig. 2). In the later stage of infection, these spots coalesced resulting in withering, extensive drying and shedding of leaves. On fruit, the symptoms started as small concentric brown sunken spots, which coalesced and covered the entire surface of the fruit. Affected fruits rotted completely (Fig. 3).

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The causal agent of this disease was successfully isolated on potato dextrose agar (PDA) from the diseased leaves and fruits. Small pieces of tissue removed from the margin between healthy and diseased tissue were surface disinfested in 1 % sodium hypochlorite for 1 min, plated on potato dextrose agar and then incubated for 7 days at 24 °C with a 12 h photoperiod. For the identification of the pathogen single spore cultures of the isolates derived, were subcultured on potato carrot agar (PCA) (Simmons 2007). These cultures gave rise to initially white colonies which turned to grayish-black later due to abundant sporulation. Conidiophores were short, septate, branched or unbranched, and green to brown. Conidia were obclavate, obpyriform or ellipsoidal with a short conical or cylindrical beak, 6.9 to 31.6 μm long and 3.5 to 23.4 μm wide at the broadest point, showing 1 to 5 transverse septa and 0 to 3 longitudinal septa. Two isolates, one from leaves and one from fruits, were deposited at the Benaki Phytopathological Institute Culture Collection (Athens, Greece) as BPIC 2692 and BPIC 2691 respectively. The morphological descriptions and measurements of the fungus are similar to *Alternaria alternata* (Fr.) Keissl. (Simmons 2007). The ITS1-5.8S-ITS2 region of four single spore isolates was amplified with primers ITS1 and ITS4 and the amplicons were directly sequenced and deposited in GenBank (Accession Nos. JX081261, JX081262, JX081263, JX081264). A BLAST search of the GenBank database revealed 100 % homology with *A. alternata* isolate JQ070079. Therefore, the pathogen was identified as *A. alternata* on the basis of its morphological characteristics and ITS sequencing.

For pathogenicity tests, three isolates obtained from diseased leaves and one isolate obtained from rotten fruits, and grown on PCA for 2 weeks, were used for artificial inoculation of loquat leaves and fruits respectively. A spore suspension of 10^6 conidia/ml was sprayed on each leaflet and fruit, while control leaves and fruits were treated the same way with

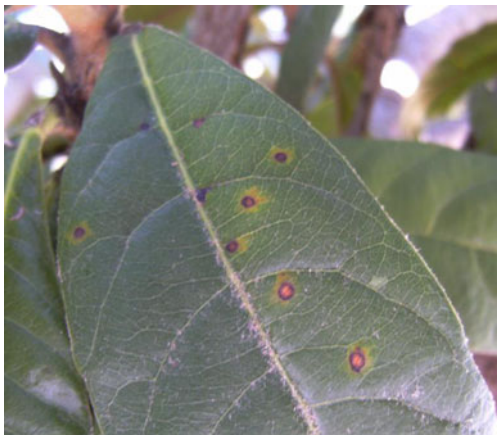

Fig. 1 Leaf spot symptoms on loquat caused by *A. alternata* (initial stage)

sterile, distilled water. Each treatment consisted of 12 leaves and fruits and the pathogenicity test was repeated three times. Leaves and fruits were then placed into 20 cm diameter petri dishes, each containing a wet sterilized piece of cotton and incubated in a growth chamber at 23 °C in the dark (80–90 % relative humidity; with a photoperiod of 12 h). Ten days after inoculation, spots similar to the original ones developed on all inoculated leaves and fruits for all four isolates (Fig. 4), while control leaves and fruits remained symptomless. *A. alternata* was reisolated from artificially inoculated leaves and fruits confirming Koch's postulates.

Fig. 2 Coalescing spots of *A. alternata* on loquat leaves

Fig. 3 Loquat fruit rot caused by *A. alternata*

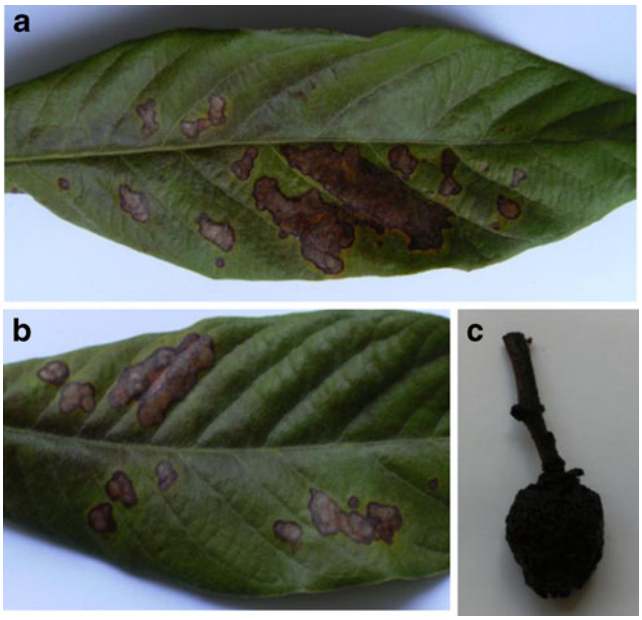

Fig. 4 Koch's postulates experiment with *Alternaria alternata* **a**, **b**. numerous necrotic spots on leaves and **c**. completely rotted fruits

There are reports of *Alternaria* sp. causing leaf spot on loquat in Florida, Japan, Mexico, Taiwan and Venezuela (Farr and Rossman 2013) and fruit rot on the same plant species in Taiwan (Ko et al. 2010). Furthermore, *A. alternata* has been reported as the causal agent of a spot disease on leaves and fruits of loquat in Palestine (Batta 2005). Nevertheless, to our knowledge, this is the first report of *Alternaria alternata* on loquat in Greece.

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