New Disease Reports

Diplodia species associated with dieback of *Retama raetam* in Tunisia

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Retama raetam is a shrub native to North Africa and is considered a medicinal plant due to its anti-microbial activity. There are a very few reports of diseases on *R. raetam* but in 2017, in the region of Bizerte (northern Tunisia) shrubs of *R. raetam* were seen with symptoms of dieback, including wilted twigs, necrosis and stem cankers.

To isolate the putative pathogen, 30 small pieces of infected stems and tissues were surface-sterilised with 3% sodium hypochlorite, washed in sterile distilled water, placed on potato dextrose agar (PDA) plates and incubated at 25°C for three days in darkness. Fungal colonies growing from the plant material were white fluffy mycelia becoming grey with age at 25°C. Within one week of subculturing semi-immersed and superficial black pycnidia formed on sterilised pine needles, placed over actively growing cultures incubated in light. Conidia were first hyaline and then turned dark brown as they matured. They were aseptate with obtuse apex and truncate ends, measuring 22.9 - 30 × 9.4 - 13.6 µm. Based on morphological and cultural characteristics, all isolates were identified as *Diplodia* sp. (Pérez *et al.,* 2010). Molecular identification was performed by sequencing the rDNA ITS region (White *et al.,* 1990), translation-elongation factor 1α gene (Carbone & Kohn 1999) and the β-tubulin genes (Glass & Donaldson 1995). One isolate TN 80 was deposited to GenBank. BLAST searches of ITS (GenBank Accession No. MN123532), EF-1-α (MN125371) and β-tubulin (MN125372) sequences revealed 98% (EU080927), 99% (EU863181) and 99% (MG015820) identity to *Diplodia sp.,* respectively.

A pathogenicity test was done according to the method of inoculation of Linaldeddu *et al*. (2014) by inoculating the pathogen on five excised healthy *R. raetam* shoots (30 cm long). A mycelial plug (3-4 mm^2) taken from the margin of an actively growing seven-day-old colony on PDA was placed in a shallow wound (3 mm) made by a scalpel on the middle of each shoot. Control shoots were inoculated with sterile PDA plugs only. The inoculated shoots were enclosed in a transparent plastic bag at room temperature (20-26°C). Four weeks post inoculation all *R. raetam* shoots inoculated with *Diplodia* showed symptoms of tip blight that appeared as brownish to black necrotic lesions extending above and below the inoculation point, stem lesions measured 53 ±5 mm. The botryosphaeriaceous fungus was successfully re-isolated from the margin of the healthy and brown lesions of each of the inoculated replicate canes whereas control shoots remained symptomless thus fulfilling Koch's postulates.

Diplodia pseudoseriata was described from native *Myrtaceae* trees in Uruguay (Pérez *et al.,* 2010). To our knowledge, this is the first report of *Diplodia* sp.causing shoot blight on *Retama raetam* in Tunisia.

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