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NEW OR UNUSUAL DISEASE REPORTS

First report of shoot blight and dieback caused by *Diplodia pinea* on *Pinus pinaster* and *P. radiata* trees in Tunisia

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Summary. Damage caused by *Diplodia pinea* on *Pinus pinaster* and *P. radiata* forests are reported for the first time in Tunisia. The affected plants show shoot blight, canker and branch dieback. On decaying and dead branches pycnidia of *D. pinea* are observed. The fungus was repeatedly isolated from the bark of symptomatic branches. The results of pathogenicity tests confirm the virulence of *D. pinea* and the susceptibility of both *Pinus* species to infection.

Key words: forest diseases, Botryosphaeriaceae, Maghreb area.

In Tunisia, coniferous forests cover 457,000 ha, which is about 55% of the total afforested area. The most important species are Aleppo pine (*Pinus halepensis* Mill.), widespread in the central and southern regions, and Maritime pine (*Pinus pinaster* Ait.), located in the north (Kroumirie-Mogods) (Anonymous, 1995). Small areas are also occupied by the exotic Monterey pine (*Pinus radiata* D. Don). Over the past century recurrent environmental injuries and high anthropogenic pressure have contributed to a progressive degradation of the Tunisian conifer forests (Ben Mansoura *et al*., 2001). Furthermore, harmful attacks of bark beetles and the occurrence of their association with plant pathogenic fungi in various pine forests have been reported (Ben Jamaa *et al*., 2007). However, little information is available about pine diseases induced by fungal pathogens.

In autumn 2006, symptoms of severe chlorosis, branch dieback, canker and shoot blight were observed in different declining stands of *P. pinaster* and *P. radiata* (Fig. 1), located in NW Tunisia in the region of Djebel Khroufa near Tabarka, and in the reserve of Aïn Zena near Aïn Draham, respectively. The symptomatic twigs of both pine species bore many erumpent black pycnidia, with dark brown conidia, oblong to clavate, aseptate, rarely one-septate, 28.9–(32.7)–39.1×10.1–(11.8)–15.1 μm, with length/width ratio of 2.8±0.02 (mean ± SE). Isolates from both conidia and affected twigs produced on potato-dextrose-agar (PDA, Fluka, Sigma-Aldrich, Milan, Italy) at 25°C an aerial and fluffy mycelium, initially white but becoming grey after 4–6 days. All morphological and cultural characteristics observed were similar to those of morphotype A of *Diplodia pinea* (Desm.) J. Kickx f. (syn. *Sphaeropsis sapinea* [Fr.:Fr] Dyko & Sutton) described by Palmer *et al*. (1987). The pure cultures derived from monoconidial isolates were maintained on PDA slants and stored at 4°C in the collection of the Department of Plant Protection, University of Sassari (accession numbers PVST 1–13). Pathogenicity tests were carried out on seven 2-year-old *P. pinaster* and *P. radiata*

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Fig. 1. Disease symptoms associated with *Diplodia pinea* infection on *Pinus pinaster* (top) and *Pinus radiata* (bottom) trees.

seedlings grown in plastic pots (10 cm diameter, 1.5 l volume) and maintained in a greenhouse for two months at 18–28°C, using two representative strains of *D. pinea* isolated in this study from twigs of *P. pinaster* and *P. radiata*, respectively. A mycelial plug (3–4 mm^2) of each strain, taken from the margin of an actively growing colony on PDA, was put in a shallow wound made with a scalpel on the stem foot of each seedling. Sterile PDA plugs were placed into similar wounds on three control seedlings of each pine species. The inoculation points were wrapped in Parafilm® for one week to retain moisture. At the end of the experimental period, the inoculated fungus was re-isolated from the margins of necrotic tissues of inoculated plants. After 4–6 weeks all seedlings inoculated with *D. pinea* showed symptoms of tip blight and dark brown discolorations of the bark and wood tissues in the stem and eventually died. Stem lesions measured 5.9±0.6 cm and 7.7±0.3 cm (mean ± SE) in *P. pinaster* and *P. radiata* seedlings, respectively (Fig. 2). Several brown erumpent pycnidia of *D. pinea* were observed on the bark around the inoculation point and occasionally on needles at the base of the canopy. No symptoms were observed in the control seedlings. The pathogen was recovered from all the symptomatic seedlings, thus fulfilling Koch's postulates. Therefore, the results of pathogenicity tests confirmed the virulence of the *D. pinea* strains obtained in this study and the susceptibility of both *Pinus* spp. to infection.

The results of this study show that *D. pinea* is directly involved in the aetiology of the decline disease observed on *Pinus* spp. in Tunisia. So, this emphasizes the need to carry out further research to evaluate the effective incidence and distribution of this dangerous pathogen within the coniferous forests in the Maghreb area. Over the last decades an increase in severe damages caused by *D. pinea* has been reported in several native and exotic pine plantations in many regions of the world (de Wet *et al.*, 2000; Flowers *et al.*, 2001; Franceschini *et al.*, 2006 ; Moret and Muñoz, 2007). Until now, in Africa the most serious losses associated with *D. pinea* were reported in exotic pine plantations in South Africa (Swart and Wingfield, 1991); in this region *D. pinea* is also known as an endophyte in healthy pine tissues (Smith *et al.*, 1996). To our knowledge this is the first report of damages caused by *D. pinea* on *P. pinaster* and *P. radiata* stands in Tunisia.

Fig. 2. Stem necrotic lesion caused by *Diplodia pinea* on *Pinus radiata* seedling (left) and an asymptomatic control seedling (right).

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