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Disease Notes

Dothistroma septosporum Identified in Greece on *Pinus brutia* and *Pinus nigra* Plantations

P. Tsopelas, Institute of Mediterranean Forest Ecosystems, Athens, Greece; **I. Barnes**, Department of Genetics, FABI-University of Pretoria, South Africa; **N. Soulioti**, Institute of Mediterranean Forest Ecosystems, Athens, Greece; and **M. J. Wingfield**, Department of Genetics, FABI-University of Pretoria, South Africa

Dothistroma needle blight (DNB) is caused by two ascomycete fungi, *Dothistroma septosporum* with a worldwide distribution and *D. pini* found in the United States, Russia, Ukraine, Hungary, and France (1). DNB has been known in Greece since the early 1980s (3) and the species responsible for the disease was reported as *D. pini*. In December 2011, needles were collected from three trees in Lagada, Thessaloniki regional unit of Central Macedonia (northern Greece), where the disease was first recorded. DNB infection seems to be limited to a valley in this area in *Pinus nigra* and *P. brutia* plantations established more than 50 years ago in an originally deciduous oak forest. Infections were observed over an area of 50 to 60 ha of pine plantations. Although the majority of pine trees were infected by the pathogen, the severity of the disease was relatively low and mortality of infected trees was not observed. Infections were limited to the lower branches in the 50-year-old trees, while on a limited number of younger trees of *P. brutia* (10 to 15 years old), infection was more severe, extending to the entire crowns of the trees. DNB does not appear to be very common in Greece. Infected needles had reddish-brown bands, usually with necrotic tips or entirely necrotic needles. Black sub-epidermal fruiting bodies (acervuli) were observed with the needle epidermis split and raised. Isolations were made from fruiting structures on needles of *P. nigra* and *P. brutia* (50-year-old trees) and *P. brutia* (10-year-old trees) after surface disinfection with 70% ethanol. Conidia from single fruiting bodies were transferred onto 3% malt extract agar (MEA) in petri dishes and incubated at 20°C. Colonies on MEA had a radial growth rate of 1.3 to 1.6 mm per week, were crustose, brown to grey-brown, and partly covered with slimy masses of conidia. The agar surrounding the colonies had a reddish color. Conidia from acervuli on the needles and the cultures were similar in shape and size, filiform, hyaline, 2 to 4 septate, and 1.8 to 2.5 (3.3) × 22 to 47 µm long. DNA was extracted from three cultures: one from *P. nigra* (CMW 37966) and two from *P. brutia* (CMW 37965, CMW 37967) using a standard phenol/chloroform method. The internal transcribed spacer (ITS) region was amplified and sequenced (1). Sequences were 100%

identical to *D. septosporum* isolates in GenBank (e.g., AY808291). In addition, up to 12 randomly selected acervuli were excised directly from the needles of each of the three trees and DNA was extracted using PrepMan solution (Applied Biosystems). The mating types of these samples were determined using species-specific mating type primers for *D. pini* and *D. septosporum* (2). All acervuli were confirmed to be those of *D. septosporum*. Both mating types were found on needles from *P. brutia* and *P. nigra*. However, the teleomorph of *D. septosporum* was not detected on infected needles.

References: (1) I. Barnes et al. For. Pathol. 41:361, 2011. (2) M. Groenewald et al. Phytopathology 97:825, 2007. (3) D. S. Kailidis and S. Markalas. Dasika Chronika 24:257, 1981 (in Greek).