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May 2014, Volume 98, Number 5
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<http://dx.doi.org/10.1094/PDIS-07-13-0765-PDN>

Disease Notes

First Report of *Diplodia pinea* on *Pseudotsuga menziesii* in Turkey

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Open Access.

Diplodia pinea is a latent, opportunistic pathogen of *Pinus* and other coniferous species, including *Pseudotsuga menziesii* (3). The fungus causes twig blight, branch cankers, tree and seedling collar rot, root rot, and can also infect cones (1). *D. pinea* has often been reported causing tip and shoot blight on various *Pinus* spp. in different parts of Turkey. During disease surveys on *Pinus* spp. carried out in May 2012 in Izmit in the Marmara Region (37°36'54"N, 31°20'00"E), typical shoot blight symptoms of *D. pinea* infection were also observed on the neighboring *P. menziesii* trees. Shoots and cones of *P. menziesii* were investigated for the presence of *D. pinea* pycnidia. Pycnidia from cones and shoots were placed on potato dextrose agar (PDA) and incubated at 23°C. Three isolates were obtained from shoot and cone samples. Identification of the pathogen was based on morphological characteristics of the conidia and by PCR of the ITS region of nuclear rDNA. Colonies on PDA were woolly, whitish at first turning black, sometimes partly or entirely turning light gray. Micromorphological characteristics of the *Diplodia* isolates were similar to those described in (2): conidia width 18.4 µm (SD ± 2.8) (range 11 to 22 µm) × length 34.0 µm (SD ± 5.3) (range 20 to 41 µm) (*n* = 100). Conidia were at first hyaline, later becoming brown to dark brown, oblong ellipsoid, bicellular with a distinct septum. To confirm the identity of the isolates, genomic DNA was extracted and the internal transcribed spacer (ITS) region of the rDNA was amplified using primers ITS1 and ITS4 (4). Amplicons were 483 bp in length (GenBank Accession No. KF372874) and shared 98% nucleotide identity with HM100285.1 and 97% nucleotide identity with JX981458.1 of *D. pinea*. Inoculation tests were performed on 2-year-old *P. menziesii* seedlings by placing mycelial plugs of three isolates obtained from pycnidia on the main stem after wounding with a cork borer. Control seedlings were inoculated with PDA plugs without mycelium. All seedlings were incubated at 24°C for 3 weeks in a climate chamber. Following incubation, the seedlings displayed dark brown-to-black discoloration, measuring on average 10.7 ± 0.6 cm, of the bark and wood tissues around the inoculation points on the stems. The pathogen was successfully re-isolated from symptomatic stem tissues, thus fulfilling Koch's postulates. To our knowledge, this is the first report of *P. menziesii* as a host of *D. pinea* in Turkey. *P. menziesii* is not endemic to Turkey and to date has a limited distribution (approximately 140 ha), but it is an important fast growing tree species for new industrial plantations.

References: (1) J. de Wet. PhD thesis, University of Pretoria, 2008. (2) J. de Wet et al. Plant Dis. 84:151, 2000. (3) G. Hausner et al. Can. J. Plant Pathol. 21:256, 1999. (4) T. J. White et al. PCR Protocols: A Guide to Methods and Applications. Academic Press, San

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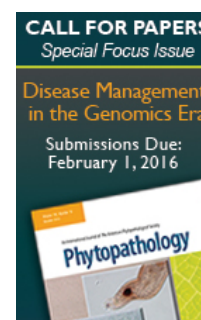
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28.12.2015

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