Plant Pathology (2010) **59**, 805 Doi: 10.1111/j.1365-3059.2009.02239.x

First report of the Dutch elm disease pathogens *Ophiostoma ulmi* and *O. novo-ulmi* in Japan

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During a survey of Japanese ophiostomatoid fungi in 2007, 91 isolates of the *Pesotum* anamorph of *Ophiostoma* were obtained from bark of fallen *Ulmus davidiana* and *U. laciniata* trees infested by *Scolytus esuriens* at Akan, Kushiro and Fujimi, Kitami in Hokkaido. Growth rate, colony morphology and mating tests (Brasier, 1981) together with sequence analyses of the ribosomal ITS region, ceratoulmin (*cu*) gene and *MAT* gene DNA, were carried out on selected isolates. On this basis, isolates from both sites were identified as the Dutch elm disease pathogens *Ophiostoma ulmi* and *O. novo-ulmi* ssp. *americana*. The mean growth rates of five *O. ulmi* and five *O. novo-ulmi* isolates at 20°C were 2·36 ± 0·31 and 3·74 ± 0·17 mm day^{-1} respectively. The sequences have been deposited at DDBJ (Accession Nos. AB519191 – 6) and isolates deposited at the FFPRI Culture Collection, Tsukuba.

During the past century *O. ulmi* and *O. novo-ulmi* have spread across Europe, North America and central Asia in two separate invasion events, causing highly destructive pandemics. Their geographic origins are unknown. Due to their considerable behavioural differences they have failed to coexist when overlapping, although transient hybrids have emerged (Brasier, 2000). This is the first report of *O. ulmi* and of *O. novo-ulmi* in Japan. There have been no previous records of Dutch elm disease in Japan (cf. Heybroek, 1982; Brasier, 1990) and no wilting of elms has been reported in the Hokkaido area. The 'sudden' finding of *O. ulmi* and *O. novo-ulmi* side by side in beetle breeding galleries therefore requires explanation. One possibility is that *O. ulmi* is endemic to Japan and *O. novo-ulmi* ssp. *americana* is a recent invasive. Genetic and field studies are in progress to assess the status and history of the two pathogens in Japan and their association with the native elms and bark beetles.

Acknowledgements

The authors thank Ms Asuka Shichiri and Mr Andrew Jeeves for technical assistance. A part of this study was funded by Global Environment Research Fund of the Ministry of the Environment, Japan (F081).

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Plant Pathology (2010) **59**, 805 Doi: 10.1111/j.1365-3059.2009.02244.x

First report of *Diplodia corticola* in Greece on kermes oak (*Quercus coccifera*)

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In 2007, symptoms of extensive branch and shoot dieback were observed on kermes oak (*Quercus coccifera*) shrubs and trees in some localities in the Messinia prefecture of south western Peloponnese, Greece. The symptoms were more intense during summer, with abundant dead branches and twigs with wilted leaves. Upon close examination, cankers were detected on the branches and twigs showing symptoms. Cankers were also evident on larger branches with no apparent foliar symptoms. In many of the branch samples taken, dark brown to black pycnidia were observed to emerge through the bark on the canker surfaces.

Fungal isolates from bark and wood tissues on symptom-bearing branches, as well as from spore masses in the pycnidia, were morphologically very similar and typical of the Botryosphaeriaceae. Colonies on malt extract agar (MEA) showed dense aerial mycelium that was initially white, gradually becoming dark grey olivaceous, with a radial growth rate of 1·3 cm day^{-1} at 25°C. Conidiomata were formed on sterile poplar twigs placed on cultures grown on water agar and incubated under lights for three to 4 weeks. Conidia formed in culture and those from pycnidia on cankers were similar in shape and size: cylindrical with rounded ends, hyaline and unicellular, becoming light brown and two-celled with age, (20-)24–32(-38) × (10-)12–16(-17) μm. Morphological characteristics and nucleotide sequences of the ITS region of ribosomal DNA (GenBank Accession Nos. GQ396149-GQ396153) confirmed the identification of the fungus as *Diplodia corticola* (teleomorph: *Botryosphaeria corticola*) described by Alves *et al.* (2004).

Pathogenicity tests were carried out in the summer of 2008 on *Q. coccifera* shrubs and trees growing in the same area as the naturally infected plants. Branches (0·7–5·0 cm in diameter) were wound inoculated with mycelium on agar plugs. Necrosis of bark and wood tissues was evident on the branches 6 weeks after inoculation, with the formation of characteristic cankers and wood discolouration beneath the cankered area. Pycnidia formed on the majority of inoculated branches. The fungus was consistently re-isolated from necrotic bark and wood tissue, as well as from pycnidial spore masses on the inoculated branches. No symptoms developed on control branches inoculated with sterile MEA. *Diplodia corticola* has been reported to occur on oak species in Spain, Portugal, Italy and Morocco (Alves *et al.*, 2004). This is the first record of *D. corticola* in Greece and *Q. coccifera* is a new host of the fungus in Europe.

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Plant Pathology