New Disease Reports

Occurence of *Boeremia exigua* var *heteromorpha* on *Nerium oleander* in the United Kingdom

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Received: *13 Oct 2012.* ***Published:*** *05 Nov 2012.* ***Keywords:*** *blight, leaf spot, fungal disease, Phoma*

In August 2010, diseased samples of oleander (*Nerium oleander*) were received from the Plant Heritage National Plant Collection located in Devon, UK. Symptoms included leaf spots, stems lesions and dieback (Fig. 1). The disease was aggressive and a threat to the collection having affected 50% of the plants. Microscopic examination revealed the presence of irregularly scattered globose pycnidia associated with the necrotic lesions. The conidia were hyaline, mainly aseptate, occasionally one-septate, and measured 5.5-8.3 x 1.2-4.4 µm (average 6.5 x 2.9 µm) (Fig. 2). A fungus was isolated on potato carrot agar (PCA) supplemented with ampicillin (30 µg/ml) and streptomycin sulphate (133 µg/ml). The mean daily radial increment was calculated based on values obtained from three isolates replicated three times. The growth rates were 8.3, 11.8 and 12.6 mm/day on malt extract agar (MA), oatmeal agar (OA) and PCA respectively at 22°C in the dark. A drop of concentrated NaOH at the margins of a 10-day-old MA culture produced a blue-green pigmentation that changed to red-brown, confirming production of antibiotic 'E' (derived from 'exigua') (Boerema *et al*., 2004) (Fig. 3). The ITS region was amplified using ITS1 and ITS4 primers (White *et al*., 1990) and sequenced (GenBank Accession No. JX467690). The sequence was identical to *Boeremia* (syn *Phoma*) *exigua* ITS sequences in GenBank (e.g. AY899262 & EU343168). There are two species of *Phoma* and related fungiaffecting *Nerium, P. glaucispora* and *B. exigua* var. *heteromorpha*. Based on the morphological and growth characteristics on agar (Boerema *et al*., 2004), sequencing and host, the pathogen was identified as *B. exigua* var. *heteromorpha.* A voucher specimen was deposited at the Royal Botanic Gardens, Kew, UK (K(M) 173517).

To induce sporulation, the fungus was grown on water agar plates with sterilised buckwheat under near UV light (16 h photoperiod) at 22°C. After seven days, pycnidia were collected and squeezed in sterile distilled water (SDW) to release the conidia. A conidial suspension (1 x 10^4 conidia/ml) was used to spray-inoculate one four-year-old *Nerium oleander* 'Pink'. Another plant was inoculated by inserting 3 mm plugs of a seven-day-old culture into a slit made with a sterile scalpel on three stems. The plugs were moistened with SDW and wrapped in Parafilm. A plastic bag was placed over all plants for 48 h to maintain high humidity and favour infection. The plants were kept in a polycarbonate growdome where the temperature was maintained at 20°C during the day and at 15°C during the night. The first leaf spots appeared five days post inoculation (dpi) on the plant that was spray-inoculated and a lesion was visible seven dpi on the stems inoculated with mycelial plugs (Fig. 4). Control plants sprayed with SDW and inoculated with sterile agar plugs did not develop any symptoms. *Boeremia exigua* var. *heteromorpha* was re-isolated from the leaf spot and stem lesion.

This is the first report of *B. exigua* var. *heteromorpha* on *N. oleander* causing leaf spot and dieback on oleander in the UK. Following this first report other cases were confirmed in West Midlands and Berkshire counties. This disease is known previously where *Nerium* is grown, including France, Italy, Spain and USA (Boerema *et al.,* 2004; Álvarez *et al*., 2005).

Acknowledgements

The author would like to thank Ian Waghorn and Jenny Denton for their technical support and Plant Heritage for funding the research into this disease.

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Figure 1

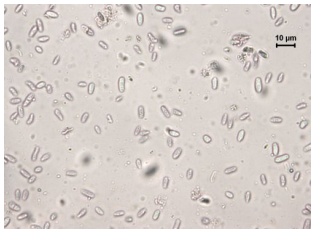

Figure 2

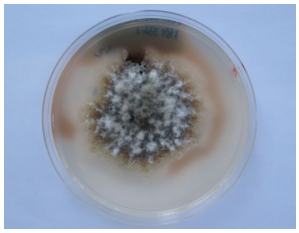

Figure 3

Figure 4

To cite this report: Henricot B, 2012. Occurence of *Boeremia exigua* var *heteromorpha* on *Nerium oleander* in the United Kingdom. *New Disease Reports* **26**, 20. [http://dx.doi.org/10.5197/j.2044-0588.2012.026.020]

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