New Disease Reports

Neofusicoccum parvum, agent of leaf spot on the new host *Ginkgo biloba* in Iran

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Ginkgo (*Ginkgo biloba*) is increasingly popular as a street, park and specimen tree in Iran. Historically, it is very ancient having fossils recognisably related to modern ginkgo from the Permian Period, dating back 270 million years ago. In September and October 2013, a moderately severe leaf spot was observed on ginkgo cultivated in some parks and green spaces in Sari, Iran (Mazandaran province). Considerable defoliation was noted with approximately 50% of the trees showing symptoms. Spots originate as water-soaked patches and then develop into irregular areas that finally are ashen in the centre with a dark reddish brown border (Fig. 1). Such spots may occur in any location on the leaf and are not limited to the radiating vascular bundles.

A fungus with grey-black colonies was consistently isolated from the leaf spots on potato dextrose agar (PDA), but produced few conidia. Abundant pycnidia and conidia developed when isolates were cultured on 2% water agar at 25°C under near-UV light for two weeks. Conidia were hyaline, ellipsoid, unicellular, with a sub-truncate base, 20–28 (24.4) x 4-6.5 (5.2) (n=50). The pathogen was identified as *Neofusicoccum parvum* on the basis of morphology (Crous *et al*., 2006). A representative isolate was characterised by sequencing of the internal transcribed spacer (ITS) rDNA using ITS4/ITS5 primers (White *et al*., 1990) and sequencing the *β-tubulin* gene (TUB2) using T1/T2 primers (O'Donnell & Cigelnik, 1997). BLAST searches of GenBank showed close identity (99-100%) of the isolate sequence to reference sequences for *N. parvum* (KC507808.1 and KC507806.1 for *β-tubulin* and KJ190288.1, KJ190287.1, KJ190286.1 for ITS). Amplified sequences from selected isolates were deposited in GenBank with the following accession numbers: KJ872493 (ITS) and KJ841779 (TUB).

Pathogenicity tests were done by inoculating each of 10 leaves on three seven-year-old trees with a mycelial plug (0.5 cm diameter) harvested from the periphery of a seven-day-old colony grown on PDA. An equal number of leaves on the same tree were inoculated with plugs of PDA medium to serve as controls. Inoculated leaves were covered with plastic bags for 24 hours after inoculation to maintain high relative humidity. The plugs were removed after 48 hours. After seven days, all of the inoculated leaves showed symptoms identical to those observed in the field under natural conditions, whereas controls remained symptom-free. Re-isolation of the fungus from lesions on inoculated leaves confirmed that the causal agent was *N. parvum*. A literature review for fungal diseases of *G. biloba* in Iran revealed only *Phyllosticta ginkgo* causing a leaf spot (Viennot-Bourgin *et al*., 1970), but no reports of *N. parvum*. However, *N. parvum* has been associated with grapevine decline in Iran (Mohammadi *et al*., 2013). To the best of our knowledge, this is first record of this pathogen as causal agent of leaf spot on ginkgo in the world.

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Figure 1

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