New Disease Reports

First report of bleeding canker caused by *Rahnella* sp. on *Populus nigra* in Iran

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In 2019, a bleeding canker with a sour-smelling and brownish liquid exudate was observed on mature (>10 years old) black poplar (*Populus nigra*) trees in eight poplar plantations in the Hamadan, Kermanshah, Isfahan, and Kohgiluyeh and Boyer-Ahmad provinces of Iran. Up to 85% of trees were affected in some stands and the trees showed a general decline and yellowing of leaves. The exudates and cankers were found on the trunk in spring and summer (Fig. 1). The symptoms were similar to bark canker of *Populus* × *euramericana* in Spain (Biosca *et al*., 2006).

Samples were collected from symptomatic trees. Fifteen isolations of bacteria were made on nutrient agar (NA; Merck, Germany) from the interface between diseased and healthy tissue based on Moradi-Amirabad *et al.* (2019). In all cases the predominant bacteria were cream, round, convex and smooth with entire margins on NA. Phenotypic tests were performed based on standard methods (Schaad *et al*., 2001). All isolates were Gram-negative, and facultatively anaerobic, positive for catalase, nitrate reduction, and hypersensitive reaction on geranium (*Pelargonium* × *hortorum*), but negative for oxidase, arginine dihydrolase, urease, lysine and ornithine decarboxylase, hydrogen sulphide and indole production, citrate utilization, and fluorescent pigment production on King's medium B. All isolates produced acid from D-fructose, D-galactose, D-glucose, D-lactose, D-maltose, D-mannitol, D-mannose, D-melibiose, D-ribose, D-sorbitol, D-trehalose, D-xylose, L-arabinose, and salicin. The isolates were phenotypically identical to members of the *Yersiniaceae*, specifically *Rahnella* spp. (Brady *et al*., 2014).

DNA was extracted from a representative isolate (P1) (Moradi-Amirabad *et al*., 2019). House-keeping genes *gyrB* and *infB* were partially sequenced (Brady *et al*., 2008) (Genbank Accession Nos. MT107177 and MT107178) and had 100 and 99.67% identity with *Rahnella* sp. strain L31-1-12, respectively. Using a neighbour-joining clustering algorithm based on the concatenated *gyrB*, and *infB* sequences, the P1 isolate clustered with *Rahnella* sp. strain L31-1-12 on a separate branch from other *Rahnella* spp. (Fig. 2). This suggests that the isolates from poplar trees in Iran belong to the genus *Rahnella* but are distinct from other named species.

Pathogenicity tests were performed as described by Li *et al*. (2014). Four strains (P1, P3, P7, and P12) were cultured on NA for 48 hr at 28°C. Bacteria were suspended in sterile distilled water (c. 10^7 CFU/ml) and 100 µl was pipetted into cross-shaped wounds (5 mm long) made in the bark of three excised stems with a sterile knife. Control stems were inoculated with sterile distilled water. Inoculated stems were enclosed with polyethylene bags and kept in darkness at 28°C. Canker symptoms with copious brownish exudate appeared seven days after inoculation (Fig. 3). The inoculated isolates were re-isolated and their identity confirmed based on phenotypic characteristics. Control stems did not produce any symptoms and no bacteria were isolated from them.

Rahnella spp. have been associated with acute oak decline symptoms (e.g. bleeding stem) on oak and hornbeam (Brady *et al*., 2014; Moradi-Amirabad *et al*., 2019). This is the first report of a *Rahnella* sp. causing a bleeding canker on poplar trees in Iran and worldwide.

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Figure 1

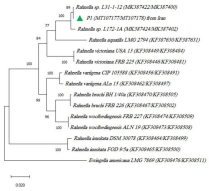

Figure 2

Figure 3

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