New Disease Reports

First report of walnut bacterial canker caused by *Gibbsiella quercinecans* and *Brenneria roseae* subsp. *roseae* in Iran

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An outbreak of a new emerging disease on walnut (*Juglans regia*) has been reported in northwestern Iran (West Azerbaijan, East Azerbaijan and Zanjan provinces) in the summer of 2018. Vertical oozing cankers were observed on trunks and main branches of diseased walnut trees especially on the lower part of the trunk, and in several cases near the crown. Principal symptoms were stem bleeding with dark exudates (brown to black resembling bitumen) and necrosis of the inner bark (Fig. 1).

Isolation of bacterial strains from infected tissues was performed on nutrient agar (NA) and eosin methylene blue agar (EMB) media. Physiological and biochemical tests were performed including Gram reaction, oxidase, utilisation of serine and citrate, lysine decarboxylase, Tween 80 dihydrolase, urease, indole production, and acid production from trehalose, arabitol, glycerol, salicin, mannitol, D-mannose, D-lactose, sorbitol, B-galactosidase, L-rhamnose, D-galactose, and arabinose. Strains were placed into three main groups similar in appearance to *Gibbsiella quercinecans*, *Brenneria roseae* subsp. *roseae* and *B. nigrifluens*. Mixed infection was not observed within a single tree during bacterial isolations. Shallow and deep bark cankers of walnut, caused by *B. nigrifluens* and *B. rubrifaciens*, respectively (McClean *et al*., 2008; Popović *et al.,* 2013), have been reported from Iran (Amirsardari *et al*., 2017).

In the present study, the pathogenicity of three main strains (Gq25, BR181 and BN142) was investigated by injecting bacterial suspensions (10^7 CFU/ml from a 48 hr NA culture) into the mesocarp of walnut fruits and under the bark of walnut branches on five-year-old walnut trees with a syringe (Biosca & López, 2012). Control samples were inoculated with sterile distilled water. Necrosis without external cankers or brownish exudates was observed on inoculated branches two months after inoculation at the inoculation sites. In the immature fruits, necrotic symptoms were observed six to eight days after injection, with necrotic tissues spread over all parts of fruits by 20 to 24 days after inoculation. To fulfill Koch's postulates, the inoculated strains were isolated on EMB medium from necrotic tissues. Bacterial strains were characterised through the partial sequencing of the 16S rRNA gene and a multilocus sequence analysis based on DNA sequence variation within two housekeeping genes (*gyrB* and *infB*). The maximum likelihood and neighbour-joining trees were constructed using MEGA7.1. The obtained sequences were compared to the reference sequences retrieved from GenBank. BLAST analysis revealed 99-100% identity with sequences of *G. quercinecans* (GenBank Accession Nos. NR117526, MG924885, CP014136 and GU562340), *B. roseae* subsp. *roseae* (KJ680128, KJ461671, KF308303 and KF308305) and *B. nigrifluens* (CP034036, NR119370, JF311838 and MH685517).

According to previous research, *G. quercinecans* and *B. roseae* subsp. *roseae* have been associated with acute oak decline in UK (Brady *et al*., 2010; Brady *et al*., 2014) with similar symptoms to the walnut disease reported here, distinguishing them from walnut cankers in Iran attributed to *B. nigrifluens* or *B. rubrifaciens*. The sequences obtained from three strains of each species, *G. quercinecans* (Gq25, Gq63, Gq89), *B. roseae* subsp. *roseae* (BR181, BR189, BR190) and *B. nigrifluens* (BN142, BN200, BN207) for 16S rRNA (MN822728-30, MN473259-61, and MN822900-2), *gyrB* (MN841613-15, MN473071-73, and MN841650-52), and *infB* (MN841621-23, MN473074-76, and MN841657-59) genes, respectively, were deposited in GenBank.

This is the first report of *G. quercinecans* and *B. roseae* subsp. *roseae* as the causal agents of bark canker on walnut trees in Iran. Further investigation is necessary to determine whether insects might be involved in the aetiology of the diseases as observed with acute oak decline.

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Figure 1

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