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Figure 1: Profuse stem bleeding typical of oak trees affected by AOD

Background

Oak decline is of complex cause, and is attributed to suites of factors that may vary spatially and temporally (Camy *et al.*, 2003; Vansteenkiste *et al.*, 2004). Often a succession of biotic and abiotic factors is involved. Two types of oak decline are recognised, an acute form and a chronic form (Vansteenkiste *et al.*, 2004, Denman & Webber, 2009).

An episode of Acute Oak Decline (AOD) currently taking place in Britain (Denman & Webber, 2009) has a rapid effect on tree health. Tree mortality can occur within three to five years of the onset of symptom development (Denman *et al.*, 2010).

Affected trees are identified by patches on stems showing 'bleeding' (Fig.1). Tissues underlying the stem bleed are necrotic (Fig.2). Larval galleries of the bark boring buprestid *Agrilus biguttatus* are usually associated with necrotic patches (Fig.3). Mature trees of both *Quercus robur* and *Q. petraea* are affected.

Few bacterial pathogens are reported to cause disease on oak. Recently a novel genus and species, *Gibbsiella quercinecans* (Brady *et al.*, 2010), were described from oak spp. with symptoms of stem bleeding in Britain and Spain. The exact role played by *G. quercinecans* in AOD is presently under investigation.

Other bacterial isolates, recovered from infected oak tissue, were tentatively identified as *Brenneria quercina* (causal agent of drippy nut disease) based on 16S rRNA gene sequencing. The polyphyletic nature of *Brenneria* prevents assigning these strains to a species using only 16S rRNA gene sequencing.

Following the successful resolution of taxonomic issues within *Pantoea* and *Tatumella* using multilocus sequence analysis (MLSA), this procedure was used on all *Brenneria* species and oak isolates from Britain and Spain to determine their taxonomic position within the *Enterobacteriaceae*.

Methods

Methods were followed according to Brady *et al.* (2008).



Figure 2: Necrotic tissue under bleeding patches on stems of AOD trees.



Figure 3: Larva gallery (↑) of *Agrilus biguttatus* and necrotic tissues (↑).



Figure 4: Adult *Agrilus biguttatus*.



Figure 5: Larva of *Agrilus biguttatus*.

Results & Conclusions

The maximum likelihood tree based on concatenated multilocus sequencing data, clearly differentiated validly published *Brenneria* spp. and closest phylogenetic neighbours, *Pectobacterium* and *Samsonia* (Fig. 6). MLSA can therefore clearly differentiate between these plant-associated genera.

The majority of British oak isolates falls within a large cluster in the *Brenneria* clade, indicating that these strains belong to a novel species, for which the name *B. goodwinii* is proposed.

A second cluster of British oak isolates groups with the Spanish oak isolates and *B. quercina* reference strains. This clade is on a separate branch far removed from the remaining *Brenneria* species. As the strains are also phenotypically and biochemically different to the other *Brenneria*, it suggests that *B. quercina* should be transferred to a novel genus.

DNA-DNA hybridization values between *B. quercina* strains and the British and Spanish oak isolates are borderline for species delineation, and the strains cannot be assigned novel species status.

Therefore *B. quercina* is transferred to *Lonsdalea*, as *Lonsdalea quercina* comb. nov.

The oak isolates from Britain and Spain are assigned to two novel subspecies, *Lonsdalea quercina* ssp. *britannica* and *Lonsdalea quercina* ssp. *iberica* respectively.

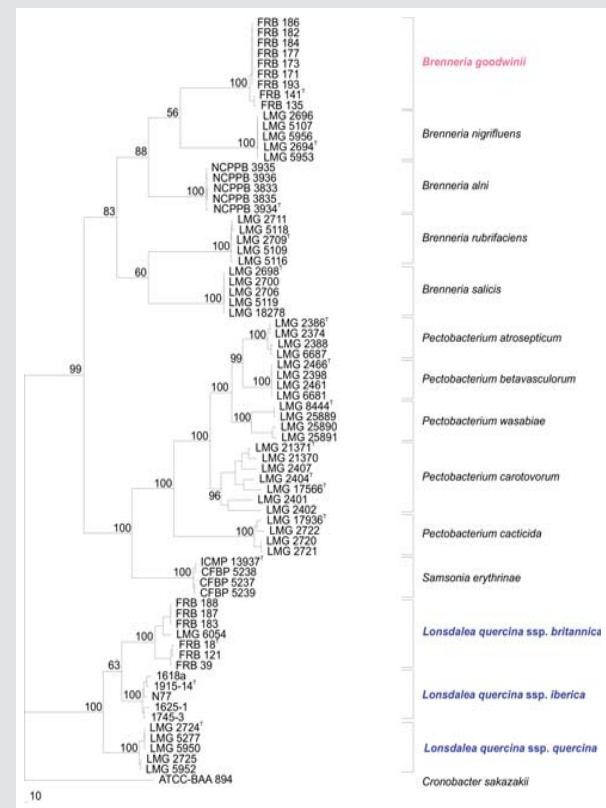


Figure 6: Maximum likelihood tree based on concatenated partial sequences of *gyrB*, *rpoB*, *infB* and *atpD* of strains from species of *Brenneria*, *Pectobacterium*, *Samsonia* and oak isolates from Britain and Spain.

References

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