


NEW DISEASE REPORT

First report of *Brenneria goodwinii*, *Gibbsiella quercinecans* and *Rahnella victoriana* in declining oaks in France

L. Eichenlaub¹  | S. Denman² | C. Brady³ | D. Maddock³ | F. Robledo-Garcia¹ | A. Aubert¹ | C. Husson⁴ | C. Robin¹

¹INRAE, Université Bordeaux, BIOGECO, Cestas, France

²Forest Research, Farnham, UK

³Centre for Research in Bioscience, College of Health, Science and Society, University of the West of England, Bristol, UK

⁴Département de la Santé des Forêts, Ministère de l'Agriculture et de la Souveraineté Alimentaire, DGAL, Paris, France

Correspondence

L. Eichenlaub and C. Robin INRAE, Université Bordeaux, BIOGECO, F-33610 Cestas, France. Email: lisa.eichenlaub@inrae.fr and cecile.robin@inrae.fr

KEYWORDS

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Acute Oak Decline (AOD) is mediated by abiotic factors (temperature and precipitation) and triggered by insects (mainly the bark-boring beetle *Agrilus biguttatus*) and a complex of bacterial species (mainly *Brenneria goodwinii*, *Gibbsiella quercinecans* and *Rahnella victoriana*) (Denman *et al.*, 2017; Doonan *et al.*, 2020). Given the extent of oak dieback and the prevalence of *A. biguttatus* in France (Saintonge & Goudet, 2020; Sallé *et al.*, 2022), a preliminary study was done in five French forests to assess the prevalence of AOD symptoms (bleeding cortical lesions on the trunk associated with insect emergence holes and dieback) and to determine the bacteria associated with those symptoms.

The mean prevalence of AOD symptoms was estimated at 37% around 20 trees assessed in each of the five forests. Bark samples and, when possible, exudates were taken from lesions associated with insect emergence holes and/or cracks (Fig. 1). Bacterial isolations were made from 43 bark samples and 11 exudate samples by plating on three agar media (Luria, Gifu Anaerobic and Eosin Methylene Blue) and incubated at 22°C for one to five days.

Bacterial strains were identified by high-resolution melting (Brady *et al.*, 2016) or 16S rRNA sequencing (Denman *et al.*, 2016). The sequenced strains had 100% identity with sequences of reference strains (GenBank Accession Nos. CP014137.1, CP014136.1 and NR_146847.1). The percentage of trees infected with *G. quercinecans*, *B. goodwinii* and *R. victoriana* was 21, 16 and 12, respectively. These AOD-associated bacteria were detected with a higher success rate in exudates (81%) than in bark (25%). *Gibbsiella quercinecans* and *B. good-*



FIGURE 1 Black exudate oozing from bark crack and emergence hole of *Agrilus biguttatus* (*Quercus petraea*, Marcenat, France).

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FIGURE 2 Lesions and necrosis on excised stem of *Quercus robur* two months after inoculation with *Brenneria goodwinii* (left), *Gibbsiella quercinecans* (middle) and *Rahnella victoriana* (right), un-inoculated control at the top of each stem.

winii were isolated in four forests, *R. victoriana* in three forests. All three species were detected on both *Q. robur* and *Q. petraea*. The co-occurrence of two species in one lesion was observed in five trees. Sequences have been deposited in GenBank (OR554234, OR554237 and OR554232).

For pathogenicity tests, excised branches (3 cm in diameter) from three actively growing *Q. robur* trees were incubated (21°C with a 16-hour photoperiod) in a jar containing water, with the upper part of the section sealed with plastic film. *Brenneria goodwinii*, *G. quercinecans* and *R. victoriana* (strains FRB141T, FRB97T, FRB225T) were applied to wounds (three replicates per stem and per tree) (Denman *et al.*, 2017) using a sterile loop charged with growth from 24-hour cultures on Luria agar medium. For control inoculations, only a wound was made. After eight weeks, cortical necrosis was apparent around all inoculation points, while only brownish discolouration was observed for

control wounds (Fig. 2). The inoculated bacteria were isolated from the necrotic tissue of the inoculated stems but not from the controls.

This is the first report of *B. goodwinii*, *G. quercinecans* and *R. victoriana* in France. Further research into the role of these bacteria in the observed oak decline and their interactions with pathogenic or endophytic fungi and other factors involved in AOD is underway.

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ORCID

L. Eichenlaub <https://orcid.org/0009-0007-1704-640X>

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