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Background:

- Decline-diseases are complex syndromes involving multiple biotic and abiotic agents, and are becoming problematic globally.
- Currently the UK is facing an episode of Acute Oak Decline (AOD), which represents a serious threat to native oak species (*Quercus robur* and *Q. petraea*).
- AOD is characterised by stem bleeds, morbid inner bark, and larval galleries of the native buprestid *Agilus biguttatus*, all of which disrupt the normal health and function of the tree.
- Several novel species of bacteria are consistently associated with AOD lesions, but the origin and pathogenicity of these bacteria is unknown.

Aims and objectives:

- The aim of this work was to identify the causal agents of tissue necrosis in AOD lesions.
- In the absence of a single potential causal agent of AOD lesions, a multifaceted approach was required using DNA sequencing technologies and infectivity studies to identify the key causal agents of necrosis within AOD lesions.

Results:

- Three bacterial species, *Brenneria goodwinii*, *Gibbsiella quercinecans* and *Rahnella victoriana* are consistently abundant in AOD lesions and possess virulence genes that are expressed in the decomposing tissues.
- Inoculation tests demonstrated that *B. goodwinii* and *G. quercinecans* cause tissue necrosis, but when combined cause more damage, and when inoculated together with *A. biguttatus*, produce the diagnostic symptoms of AOD.

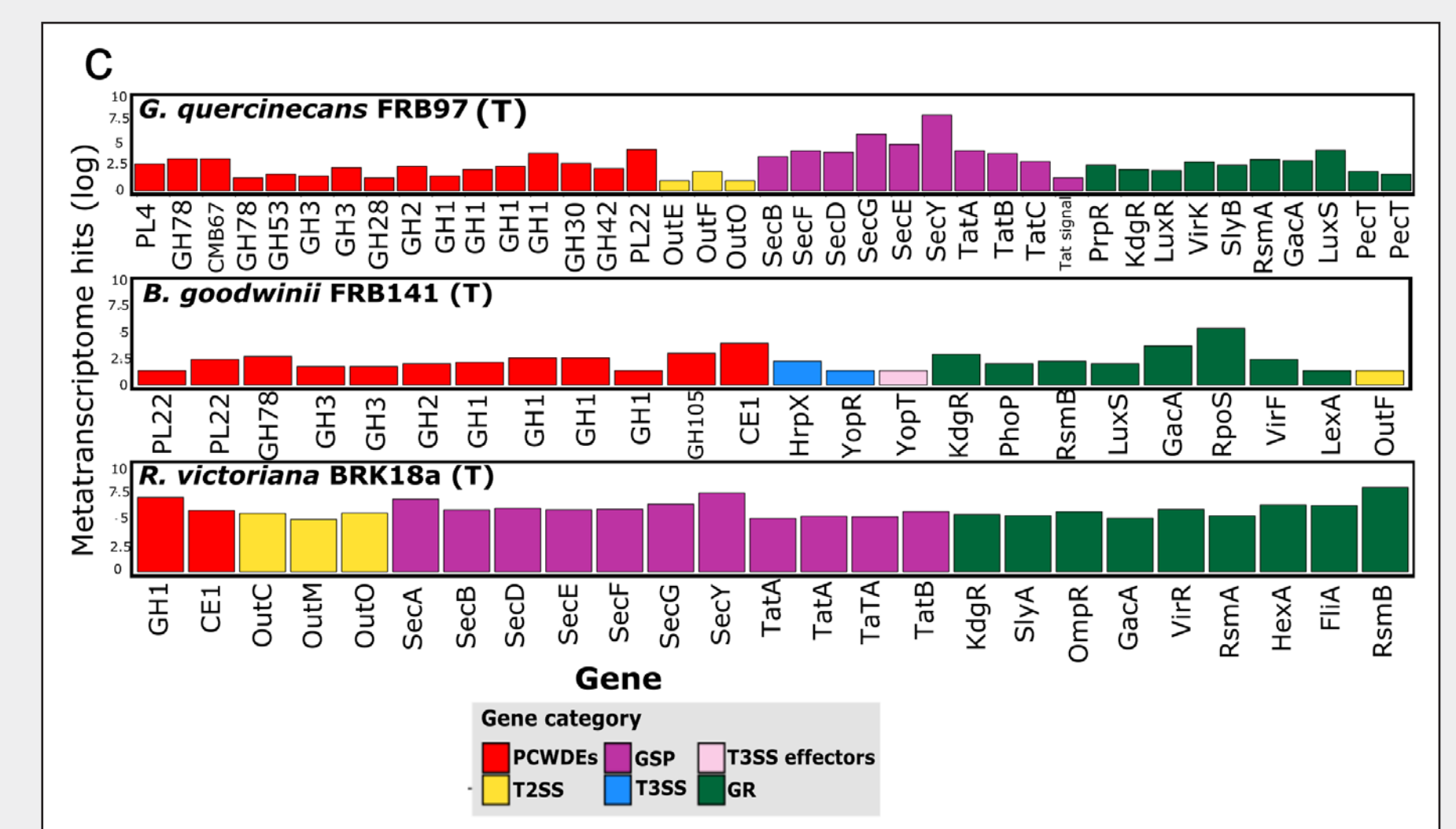
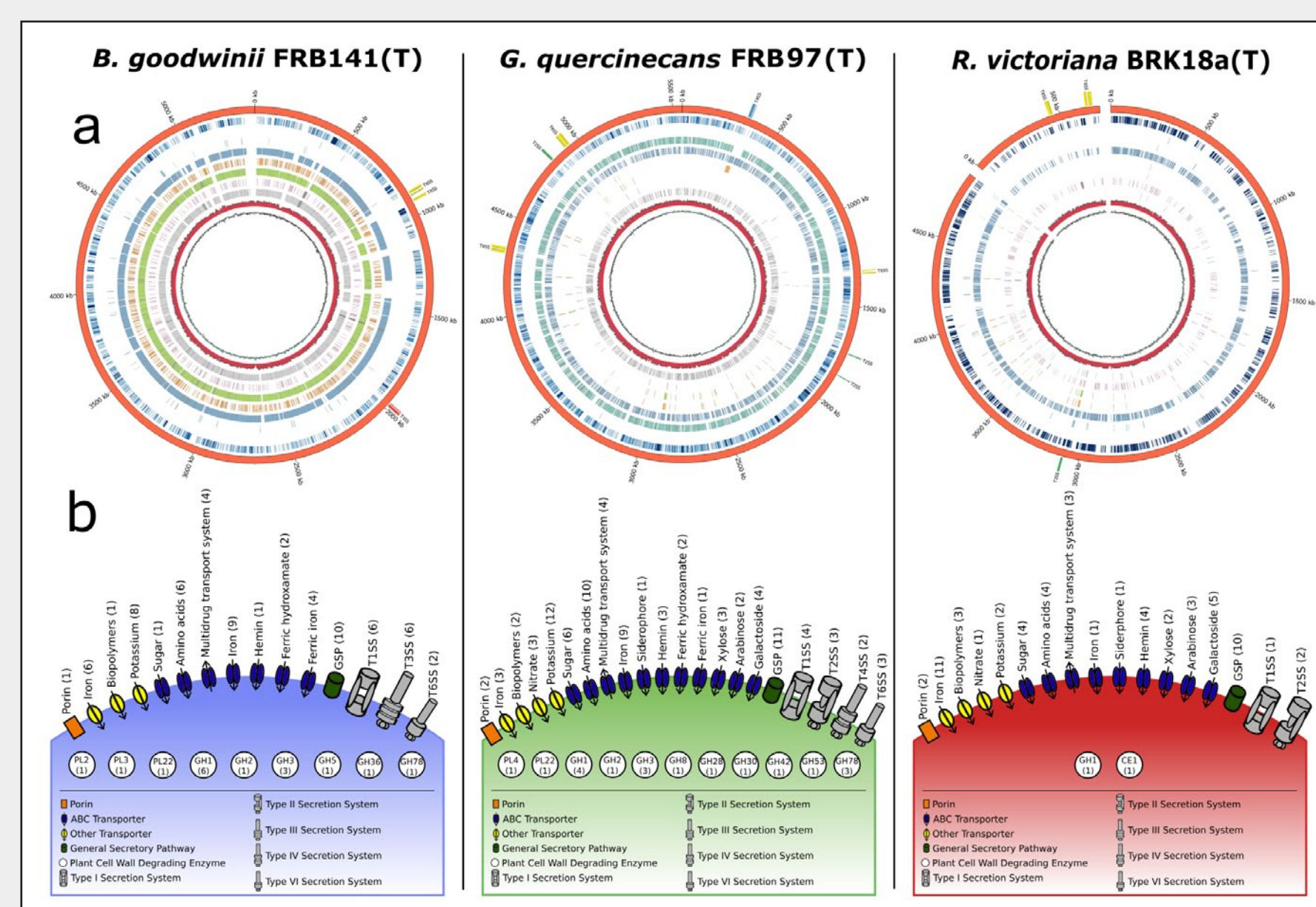


Fig. 2. (Left) Genome sequences of bacteria consistently associated with AOD lesions indicating presence of pathogenicity and virulence genes
Fig. 3. (Right) Expression of bacterial pathogenicity and virulence genes in AOD lesions

Fig. 4 a-h. Infectivity tests demonstrating bacterial lesion formation and interaction with *A. biguttatus* larvae

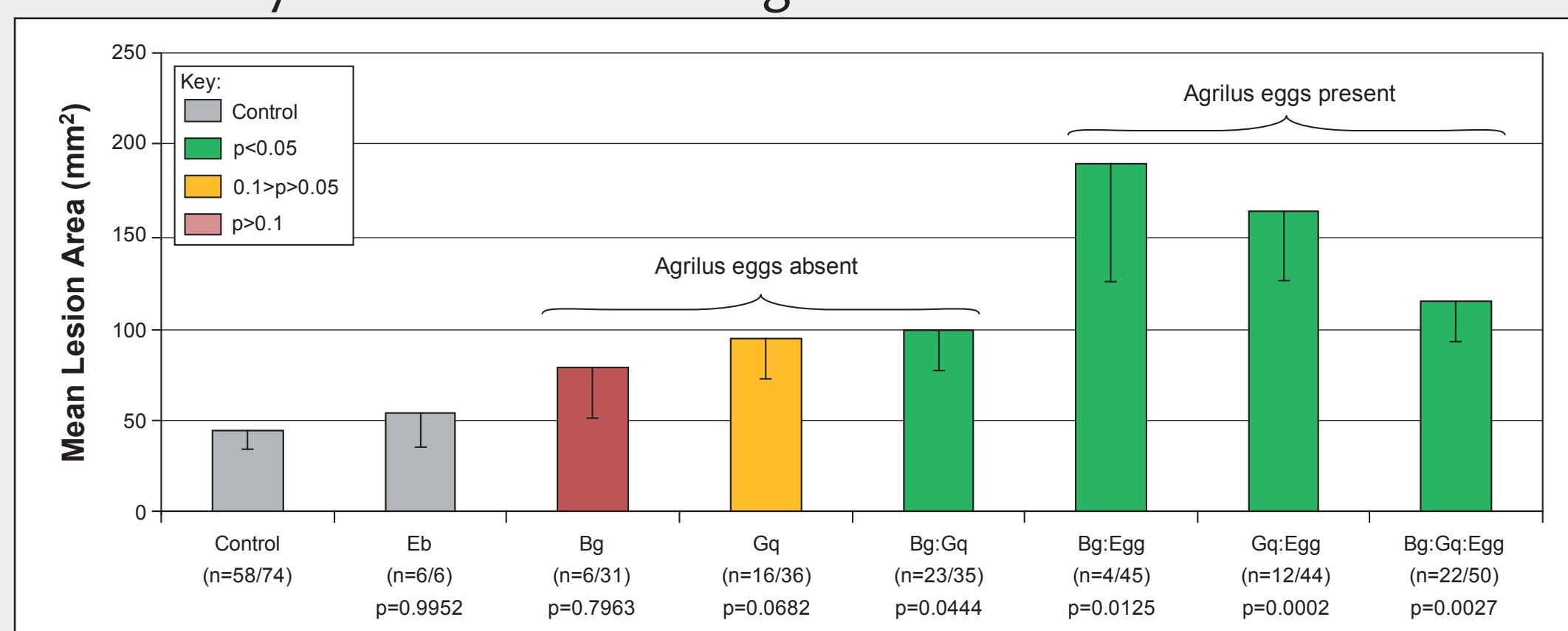


Fig. 4a. Mean lesion area caused by bacterial strains with or without larvae of *A. biguttatus* (Eb = *Erwinia billingiae*, Lqb = *Lonsdalea quercina* ssp *britannica*, Bg = *Brenneria goodwinii*, Gq = *Gibbsiella quercinecans*). Larvae were derived from *A. biguttatus* eggs produced *in vitro* and applied to log tests as egg clusters or as individual day old larvae.

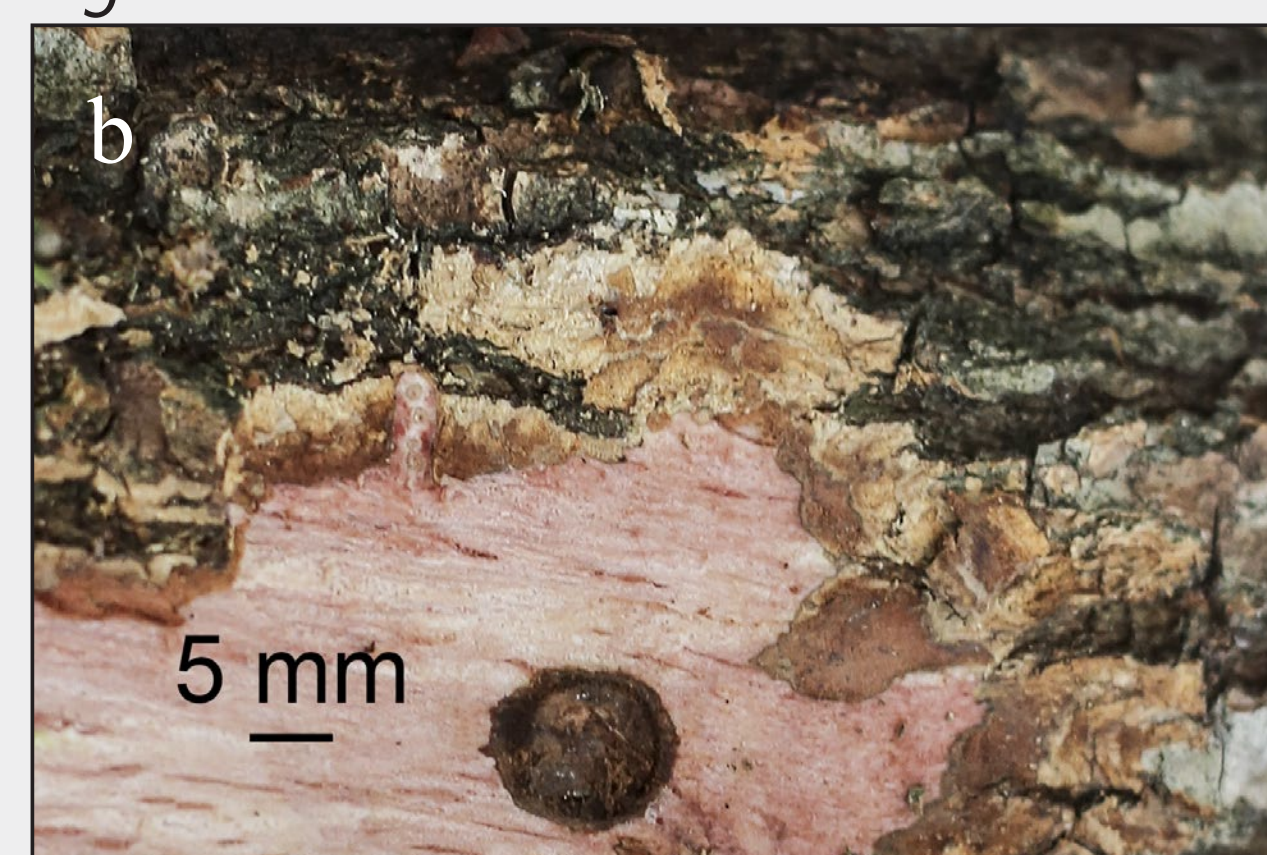


Fig. 4b. Wound response in wounded control treatment



Fig. 4c. Tissue necrosis caused by *Brenneria goodwinii*

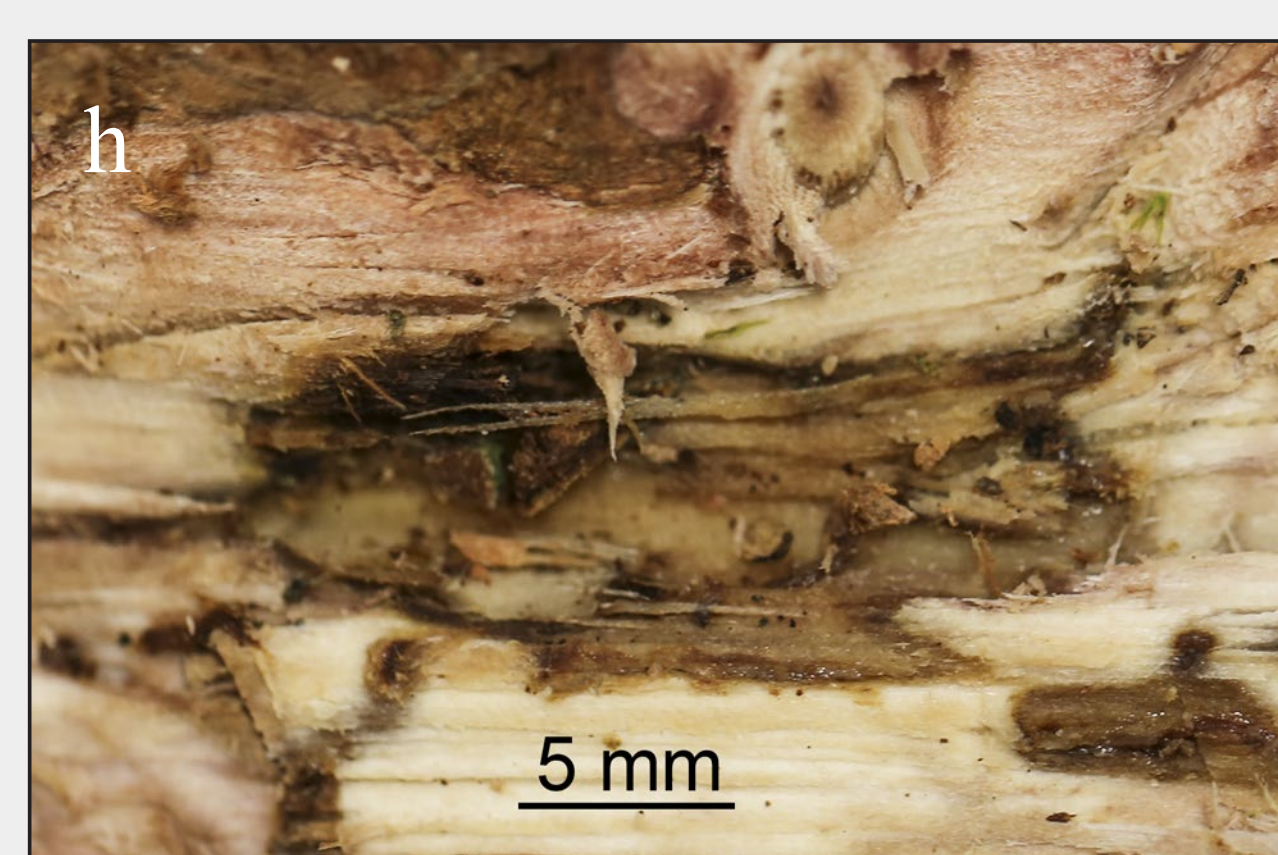
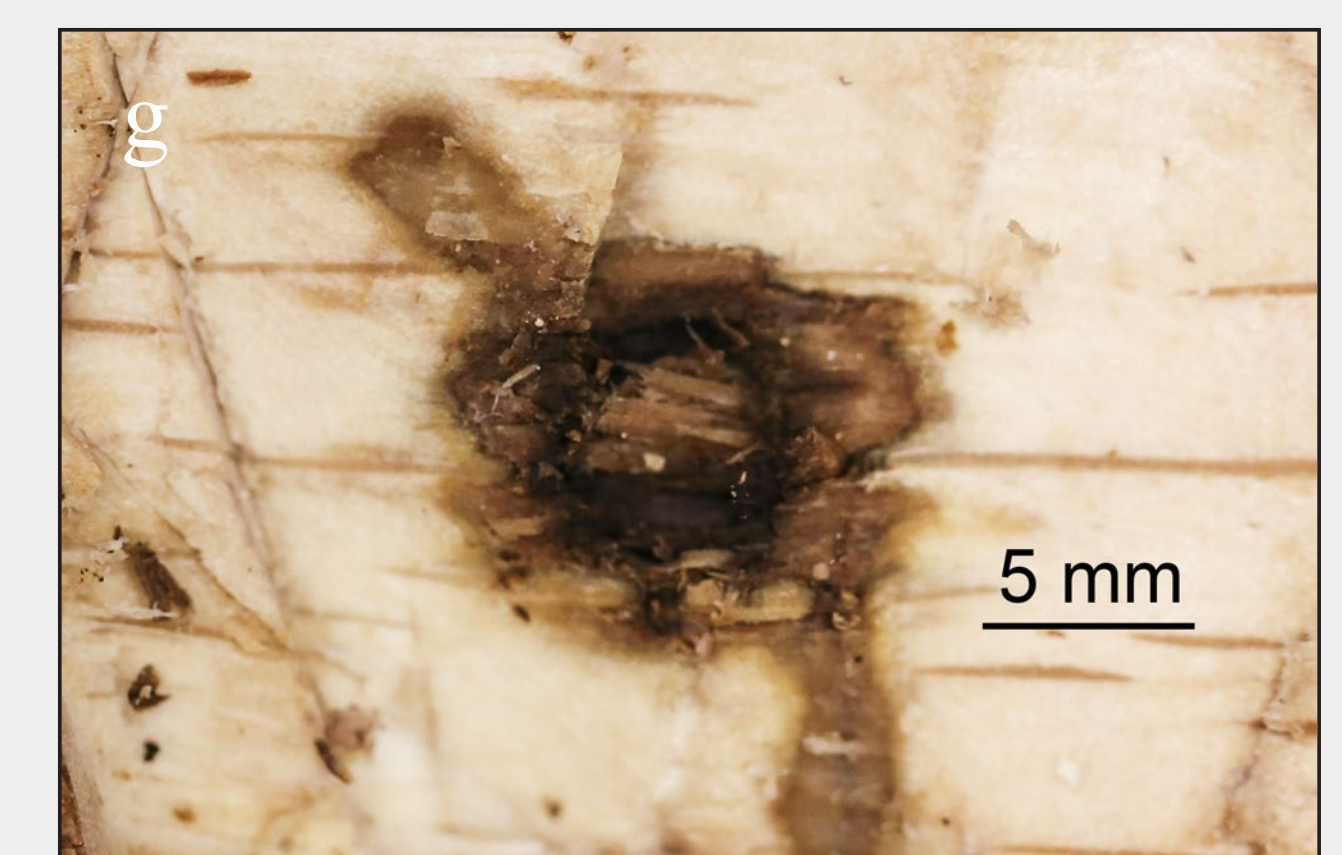
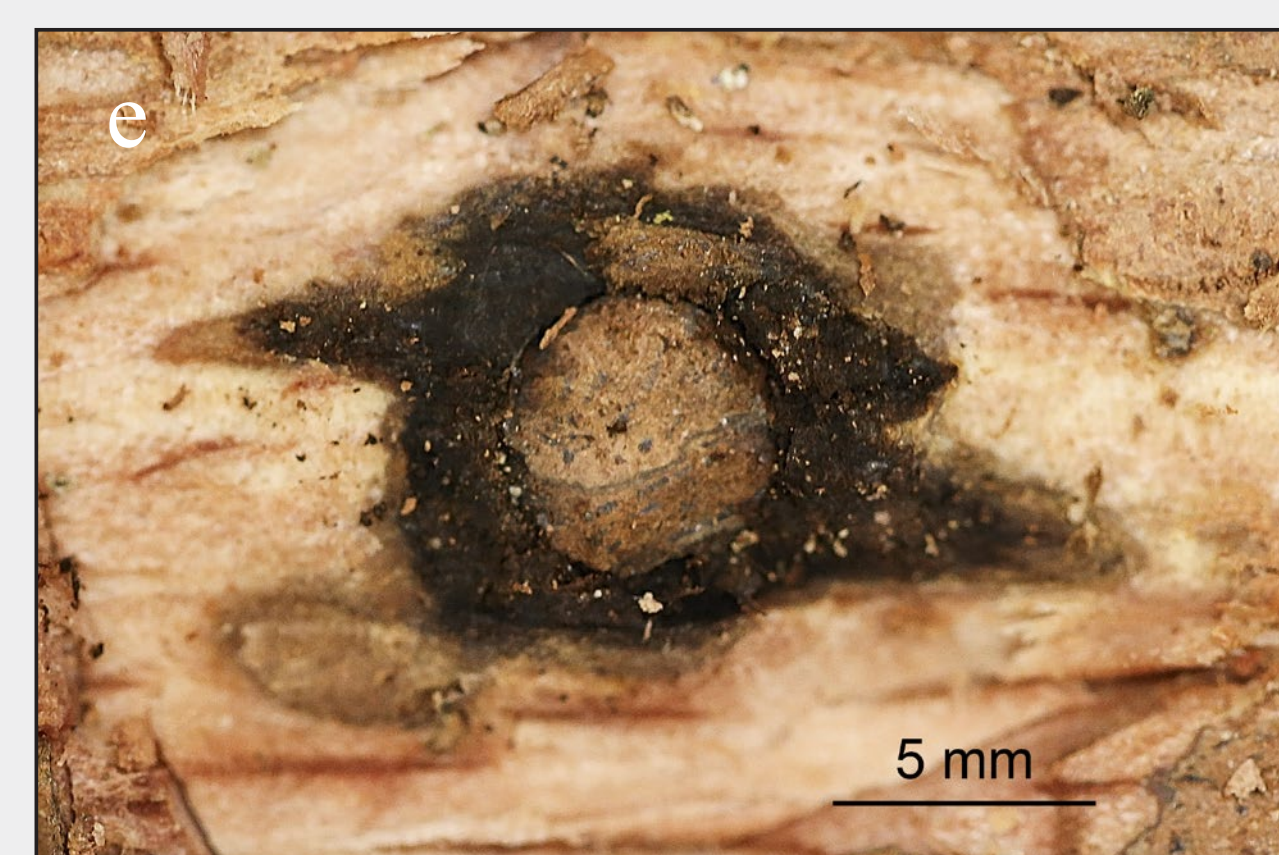


Fig. 4d. Tissue necrosis caused by *Gibbsiella quercinecans*
Fig. 4e. Tissue necrosis caused by a mixture of *Brenneria goodwinii* and *Gibbsiella quercinecans*
Fig. 4f. Tissue necrosis caused by *Brenneria goodwinii* and *Agilus biguttatus* larvae
Fig. 4g. Tissue necrosis caused by *Gibbsiella quercinecans* and larvae of *Agilus biguttatus*.
Fig. 4h. Tissue necrosis caused by *Brenneria goodwinii*, *Gibbsiella quercinecans* and *Agilus biguttatus*.

Conclusions:

- AOD lesions are caused by a polybacterial complex, providing novel insights into polymicrobial interactions in tree disease.
- The prevailing paradigm that plant diseases are caused by a single pathogen (Koch's postulates) requires contemporary adaptation for complex polymicrobial diseases.
- We present a novel conceptual and methodological template to address the role of microbial communities in disease in future studies of plant health.

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