

A Polyphasic Approach to Gaining Insights Into Causes of Acute Oak Decline in Britain¹

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Abstract

Acute Oak Decline (AOD) is a complex disease syndrome of native oak, making its first appearance in the 1980s in Britain. Since then increasing reports of its occurrence have raised concerns about cause and effects on these iconic trees. Symptomology studies confirmed that AOD is a distinctive condition with four diagnostic symptoms defining the condition; (a) weeping patches on trunks of affected trees, (b) cracks between the bark plates, (c) inner bark necrosis and sapwood degradation underlying the weeping patches, (d) over 95% co-occurrence of larval galleries of a native bark boring buprestid, *Agilus biguttatus*, in the cambial tissues. A polyphasic approach encompassing landscape to molecules, to determine possible causes of the syndrome has been adopted. The microbiomes of healthy and symptomatic trees were compared using: (a) conventional methods, (b) metabarcoding of the V3-V5 fragment of the bacterial 16S rRNA gene and the fungal ITS gene region, (c) 454 pyrosequencing of total metagenomes, and (d) pyrosequencing the metatranscriptome (rRNA and mRNA) of the lesions on AOD affected trees. Microbiome analyses supported isolation data, which showed a shift from healthy to diseased trees, with members of the *Enterobacteriaceae* dominating the lesions. Two novel species, *Brenneria goodwinii* (*Bg*) and *Gibbsiella quercinecans* (*Gq*), were consistently associated with necrotic tissue, suggesting a role in lesion formation, but other species such as *Lonsdalea quercina* ssp. *britannica* and *Rahnella victoriana*, which was present in both healthy and diseased trees, may also play a role. Recognition of the involvement of multiple agents led to the hypotheses that (a) bacteria, particularly *Bg* and *Gq*, have a role in causing degradation of oak phloem and sapwood, (b) interaction between *A. biguttatus* and these bacteria lead to typical AOD symptoms, (c) AOD is a complex Decline disease dependent upon the interaction of multiple factors for disease establishment. The methods used to determine the necrogenic capability of the bacteria include: (a) host inoculation tests, (b) pathogenicity assays using wild types, knock-out mutants and visualizing green/red/yellow fluorescent transformed bacteria, (c) whole genome sequencing to determine pathogenicity and virulence genes, (d) metagenomics to characterize taxonomic and functional abundance of organisms present in healthy and diseased trees and (e) transcriptomics to extract expressed virulence genes from the polymicrobial community, and to analyze gene expression of *Bg* and *Gq* *in vitro* alongside phloem and sapwood, (f) metaproteomics to provide functional evidence of gene activity. In order to investigate the possible involvement of *A. biguttatus* in necrosis, larvae were reared in the laboratory and used in inoculation studies. Preliminary results of host inoculation tests show that bacteria cause necrosis in logs, but interaction between bacteria and larvae simulate all four symptoms of AOD. Pathogenicity assays indicate differing HR ability of the various bacterial species, but changes in necrogenic ability in mixed bacterial species inoculations occurred. Genome and metatranscriptome sequencing of *Bg* and *Gq* revealed encoded pathogenicity factors, with genes for plant cell wall degrading enzymes and secretion systems, furthermore transcripts aligning to these were recovered from an active lesion. Transcriptomic and proteomic work is ongoing but to date the evidence accumulated is highly supportive of a biotic cause of the disease with key roles for *A. biguttatus* and the named Enterobacterial bacteria.

¹ A version of this paper was presented at the Sixth Sudden Oak Death Science Symposium, June 20-23, 2016, San Francisco, California.

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