

Preliminary evidence that Acute Oak Decline bacteria could be widespread in oak canopies

Researchers at Royal Botanic Gardens Kew have new evidence that the bacteria found in acute oak decline lesions are also found in the canopies of oak trees, even in healthy trees.

Many will be familiar with the problem of acute oak decline (AOD) and the bacteria that have been identified in the lesions on the trunks of oak trees. Using data taken from a whole genome sequencing project, PhD student Louise Gathercole found genetic evidence for low levels of these bacteria in the canopies of oaks at five sites in England. She did not find any differences between healthy and symptomatic trees, suggesting that the bacteria may be a part of the normal oak microbiome.

Acute oak decline seems to be caused by a complex combination of environmental stress, and the activity of bacteria and a wood boring beetle. So far, the AOD-associated bacteria have been isolated mainly from the trunks of the trees, with some early-stage research hinting that they may be found elsewhere. However, it is unknown whether these bacteria are widespread outside of lesions. Researchers at Kew and Forest Research had recently sequenced the whole genomes of oak trees (see <https://doi.org/10.1002/ppp3.10229>) from the canopy at sites monitored for AOD or chronic oak decline (COD). When DNA is extracted from a leaf, the DNA of the tree and also the DNA of microbial lifeforms that live on the leaf. Usually in a population genomics study, the sequenced 'reads' that do not belong to the main organism are discarded. The team therefore wanted to see whether these sequences could be used to detect the AOD-associated bacteria in the leaves of the trees and if there were differences between healthy and unhealthy trees, or between the different geographical locations.

Louise used a software called 'kraken', which compares short sequences of DNA with similarly short sequences in a database of microbial organisms and uses this to give the reads a label at a level from 'domain' (such as bacteria), all the way down to species level if the sequence is different enough from other genomes. Looking at all of the classifications, she did not find any differences, but a large proportion of the reads were classified as 'proteobacteria', which is the group that the AOD bacteria belong to.

Included in the microbial database were the whole genomes of the AOD bacteria (previously sequenced by AOD researchers). Using the search results, Louise found genetic evidence at species level for the AOD bacteria on the leaves of the sampled trees, but at very low levels. This evidence was found on trees with AOD symptoms, but was also found on healthy trees, those with COD and those that were recovering from AOD. She also compared the different health categories and the geographical sites in terms of the numbers of reads classified as AOD bacteria. There were differences in geographical sites, but no differences between the healthy and symptomatic trees. Clearly AOD is more complicated than a straightforward bacterial infection and hopefully this work will help to inform some of the ongoing AOD studies.

Louise Gathercole's PhD is funded by Defra and is part of the Action Oak initiative. She is based at RBG Kew and Queen Mary University of London and supervised by Prof. Richard Buggs and Prof Richard Nichols. The sampling of trees used in this research was overseen by Dr Sandra Denman of Forest Research and Dr Nathan Brown of Woodland Heritage. The paper reporting this work can be found in the journal *Forests* here: <https://www.mdpi.com/1999-4907/12/12/1683>.