Rapid detection and quantification of bacteria associated with Acute Oak Decline

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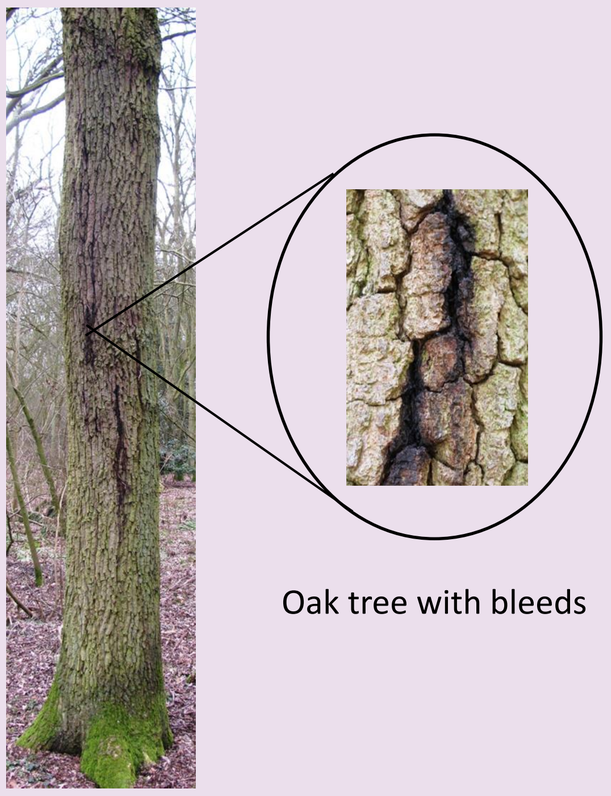
Introduction

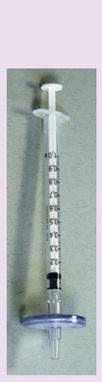
Acute Oak Decline (AOD) is a syndrome affecting mature oak trees and is characterised by stem bleeds from vertical fissures on trunks, and inner bark necrosis caused by a polybacterial consortium, in which *Gibbsiella quercinecans* and *Brenneria goodwinii,* and to a lesser extent *Rahnella victoriana* and *Lonsdalea britannica* play key roles (Denman et al., 2018). Here we report a novel multiplex real-time PCR assay that enables simultaneous rapid detection and quantification of these four bacterial species from swabs taken from stem bleeds. The assay was designed to be non destructive due to the intrinsic value of mature and veteran oak trees that are affected by AOD.

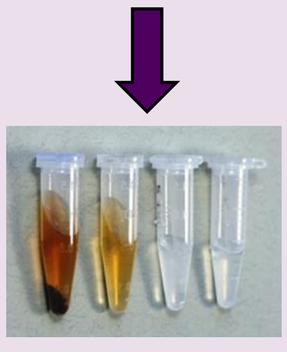
Detection Method



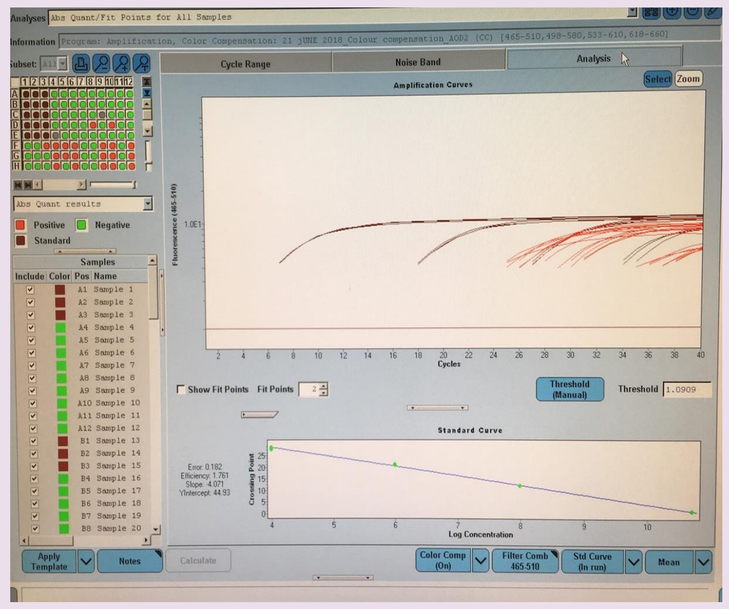
Oak tree with bleeds

Swab samples stem of exudate





Swab samples are washed, filtered and centrifuged to remove polyphenolics and collect bacteria

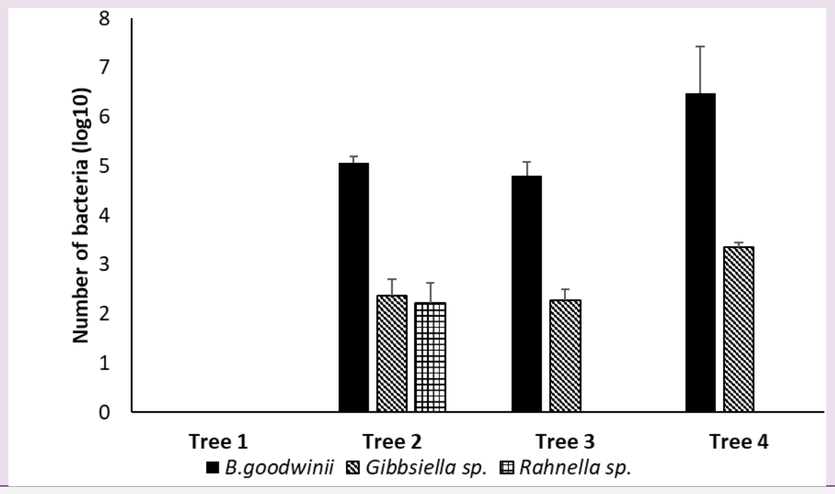


TaqMan real-time PCR (Schaad & Frederick, 2002) to detect AOD associated bacterial species. Each bacterial species was detected with a gene probe labelled with a specific fluorescent dye. The number of bacteria collected from a swab sample could be determined from a standard curve.

Detection of AOD associated bacteria in *Quercus robur* trees

Decline symptoms on *Quercus robur* trees at a site in south east England. (a) Tree 1 displaying dieback of upper branches. (b) Tree 2 and (c) Tree 3 with numerous bleeds along the length of the stem. (d) Tree 4 with a bleed at the base of the stem.

Detection and quantification of Acute Oak Decline associated bacteria on oak trees at a field site in south east England. *B. goodwinii* and *Gibbsiella sp.* were detected in three of the four trees. *B. goodwinni* was present at higher levels in the exudates than *Gibbsiella* sp. Tree 4 had the highest numbers of *B. goodwinii* (2.7×10^7) and *Gibbsiella* sp. (2.36×10^3) cells. *Rahnella* sp. was only present in Tree 2. Tree 1 had a dry stem bleed and no Acute Oak Decline associated bacteria were detected.



Conclusions

- The multiplex real-time PCR method can simultaneously detect all four of the bacterial species associated with AOD tree stem bleeds.
- The protocol is non-destructive and enables bacterial detection directly from stem bleeds without the need for removal of inner bark panels from high commodity trees.
- The primer/probe set for *B. goodwinii* was species-specific, but primer/probe sets for the other three species were able to identify other members of their respective genera.
- There was no cross detection of genera within the multiplex qPCR reaction, and non-target bacteria were not detected.
- Absolute quantification of the bacteria from swab samples was possible through the inclusion of a standard curve prepared from dilutions of gene copy standards.
- Application of the assay will greatly assist diagnostics and management of Acute Oak Decline in woodland areas.

Literature cited

Denman S *et al.*, 2018. *The ISME Journal* **12**, 386-99.

Schaad NW, Frederick RD, 2002. *Canadian Journal of Plant Pathology* **24**, 250-8.

Acknowledgements

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