What Does it Take to Stop Bad Bacteria on Oak?

Sandra Denman and **Nathan Brown** provide an update on the multidisciplinary research under way at Alice Holt into the pathology and management of acute oak decline.

Tree health has come into focus more sharply over the past decade as the detrimental impacts of pest and disease outbreaks on native and commercial forest tree species are being seen first-hand across the UK landscape. Two main mechanisms are causing an increase to pest and disease threats: the arrival of alien species facilitated by the global plant trade (Brasier, 2005, 2008; Santini et al., 2018); and climate change, which alters the environment, impacting tree growth as well as the suitability for pest and pathogen development and dispersal (Choat et al., 2018; Seidl et al., 2017). In the coming years we expect to see changes to the distribution, occurrence and severity of many arboreal pests and diseases. Acute oak decline (AOD) is one of the diseases that we expect will be aggravated by climate change. Here we give an update on current research on AOD and its management that should contribute to reducing the expected impacts of climate change on the disease.

AOD – symptoms, causes and UK distribution

Acute oak decline is an emerging disease affecting the stems of oak trees. On the outer bark black, vertically arranged, weeping stem lesions (Figure 1) and, in at least one third of cases, D-shaped exit holes of the adult native two-spotted oak buprestid *Agrilus biguttatus* (Figure 2) are distinguishing features (Brown et al., 2015; Denman et al., 2014). In the inner bark larval galleries and bacterial lesions are visible close to the cambium (Figure 3). Decay within the stem is caused by a bacterial pathobiome (multiple bacterial species that act together) (Denman et al., 2016; Sapp et al., 2016). At least three species of bacteria are involved in AOD, primarily *Brenneria goodwinii*, supported by *Gibbsiella quercinecans* and *Rahnella victoriana* (Broberg et al., 2018; Doonan and Denman et al., 2019). Decline diseases like AOD are different from primary diseases (such as ash dieback) as they result from a complex interaction between environmental stress factors, insects and microorganisms that combine to cause premature deterioration of tree health (Manion, 1981).

Adult *A. biguttatus* are bronze-green beetles with a white spot on each wing case. They are approximately 11mm long with a slender tapering appearance. Adult females lay eggs on the bark of mature oaks and are thought to favour trees already in a weakened state. It is thought that *Agrilus* beetles cannot colonise healthy oak trees (Vansteenkiste et al., 2004). Before the advent of AOD in the UK the oak jewel beetle (*A. biguttatus*), which is native to the UK (and Europe), was considered an insect responsible for 'finishing ailing/dying trees off' by girdling the tree through the larvae feeding on the live inner bark tissues. Larvae feed and develop over two years (Reed et al., 2018). As they chew through the inner bark they form tunnel-like galleries. After pupation adult beetles leave the bark through distinctively D-shaped emergence holes, which are perfectly flat on one edge and curved on the other like a capital 'D'. These holes can appear in any orientation (Figure 2).

Figure 2. D-shaped exit holes of the adult native two-spotted oak buprestid Agrilus biguttatus. (Photo: Nathan Brown FR)

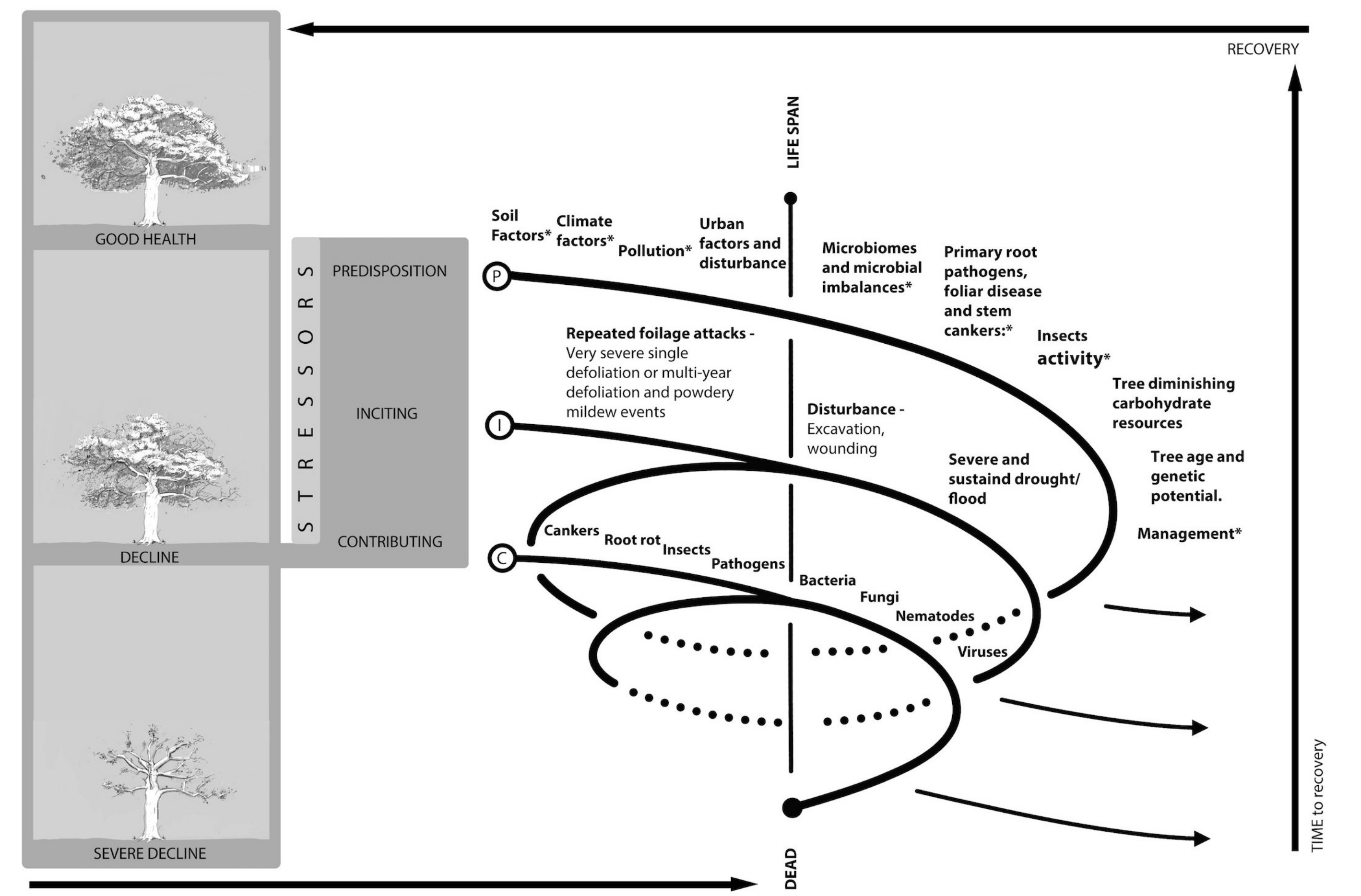
Figure 1. Weeping stem lesions characteristic of acute oak decline: Left - severe infection; Right - close-up of two weeping lesions. (Photo: Nathan Brown FR).

To date AOD affected trees are found in southern England, from the East Anglian coast to the Welsh borders, and no further north than southern Yorkshire or the edge of Lancashire (Brown et al., 2017). Analysis of the distribution of affected sites has revealed correlations with environmental variables likely to cause sub-optimal growth for oak trees. AOD has strong links to areas with low rainfall, high temperatures and where deposition of nitrogen oxides is high (Brown et al., 2018). It is therefore likely that these factors are crucial in the initial weakening of the trees, and this is an active area of current research. This process of predisposition is thought to play a crucial role in decline diseases where it reduces tree vigour and allows subsequent infestation/infection by pests and diseases that would otherwise be repelled by host defences. Drought stress in changing climates is thought to be an increasingly important predisposition factor for oak (Desprez-Loustau et al., 2006; Haavik et al., 2015). However, this is not a one-way progression and reassuringly, trees with AOD stem lesions do not always die; up to 40% of monitored trees produce callus tissue over the decay and enter remission (Brown et al., 2016). The number of trees that recover varies between years, which indicates that weather patterns (particularly periods of drought, which may limit recovery) affect resilience of oak trees, although the underlying conditions for predisposition are likely to remain present

Figure 3. In the inner bark, larval galleries and bacterial lesions are visible close to the cambium. (Photo: Sandra Denman FR)

over long time periods (Reed et al., 2020). Monitoring the underlying health of the nation's oak trees is therefore a crucial step in understanding the drivers of predisposition and decline.

The process of tree decline diseases was conceptualised by Manion, who defined a spiral model to describe the how different types of agents act together to reduce tree health. We updated and enhanced this model (Figure 4) (Denman et al., 2022). In this model predisposing factors act on otherwise healthy host trees and form the first stage of decline. Weakening may then be further impacted by one-off events or inciting factors such as extreme heat or storms. Finally, susceptible hosts are now in a weakened condition where the biotic contributing secondary agents (pests and diseases) can establish and further reduce the health of the tree.



Soil Factors:	Climatic Factors:	Pollution:	Microbiomes and microbial imbalances:	Primary root pathogens, foliar disease and stem cancers:	Insect activity:	Management:
Compaction Low moisture holding capacity Poor draining Fluctuating water table Soil acidity Poor fertility Nutrient imbalances	Low rainfall High temperatures Flooding Extreme events Frosts	Excess atmospheric and soil pollutants e.g. nitrous oxides Imbalances of nutrients e.g. P shortage	Rhizosphere soil – imbalances in bioconversion of nutrients (e.g. Nitrifying microbes). Disruption to biotransfer of nutrients (e.g. *mycorrhizae*). Disruption to protective and growth promoting activities	Buttress root rots (e.g. *Phytophthora* , *Gynmopus, Armillaria*) Feeder root rots (e.g. *Phytophthora, Pythium, Ilyonectria*) Foliar – Powdery mildew - repeated outbreaks	Repeated Insect attacks	Stock on land Planting density Thinning Mixtures Social pressures (e.g. foot fall, pathways)

Figure 4. Updated Manion 'decline disease spiral' model showing the predisposition stages and factors in the Decline process. (Denman et al., 2022). (Reprinted from Denman, S., Brown, N., Vanguelova, E. & Crampton, B. 'Temperate Oak Declines: Biotic and abiotic predisposition drivers.' In F. Asiegbu & A. Kovalchuk (eds.), Forest Microbiology (pp. 239-263), 2022, with permission from Elsevier.)

Research funding won

In 2020 our research group was thrilled to be granted a large research project on advancing knowledge and management of acute oak decline (AOD). The project forms part of a research initiative on bacterial plant diseases (BPD). It is funded by the UKRI (UK Research Innovation), which is the national funding agency that invests in science and research in the UK. Nine projects were funded, including two complimentary projects on oak: Bac-Stop (stop bad bacteria on oak) led by Forest Research; and Future Oak headed by Professor James McDonald (Birmingham University).

Bac-Stop has four main research questions investigating: the role of drought in predisposition; links between the AOD bacteria and *Agrilus* species; public and landowner opinions regarding disease management; and bacterial decay on other tree species. Future Oak is characterising the microorganisms associated with oak (the 'microbiome') and selecting advantageous organisms that can be used to counter disease and promote resilience. More information is available at https://www.bacterialplantdiseases.uk/.

Figure 5. Beetle emergence cages housing slabs of oak from which adult Agrilus biguttatus beetles will emerge in warm conditions. (Photo: Katy Reed FR)

New areas of research

Our work in the Bac-Stop project investigates whether or not *A. biguttatus* is essential to the development of tissue degradation in stems of trees affected by AOD. Previously we stated that the potential role of *A. biguttatus* in AOD is controversial and difficult to prove definitively (Reed et al., 2021). This is partly because it is extremely difficult to get these elusive beetles in hand. We are now rearing the beetles in captivity to study their potential role as vectors of AOD bacteria. To achieve this we source infested bark material from landowners and forest managers during the winter and place it in emergence cages (Figure 5). Beetles emerge in June and July (when the temperatures are warm enough) and are quickly moved to smaller breeding cages (Figure 6). After a period of feeding, mating occurs and the females lay eggs, which hatch to produce 1st instar larvae.

To find out whether the beetles are carrying, or are able to carry, bacteria four treatments are investigated: (a) newly emerged beetles are tested before they have had a chance to feed; (b) beetles are fed on 'clean' oak leaves; (c) dipping clean leaves into known AOD bacterial solutions and feeding them to the beetles; and (d) feeding beetles on natural forest leaves. We extract DNA from the beetles in all four treatments to profile the bacteria present. This work was pioneered in 2020 by Dr Michael Crampton (FR) and is being refined by Megan Richardson (FR) who joined us in July 2022 and has already dissected 180 beetles (Figure 7). Preliminary results (Crampton, 2020) showed that:

Figure 6. Beetle breeding cages used to rear Agrilus biguttatus in captivity. (Photo: Megan Richardson FR)

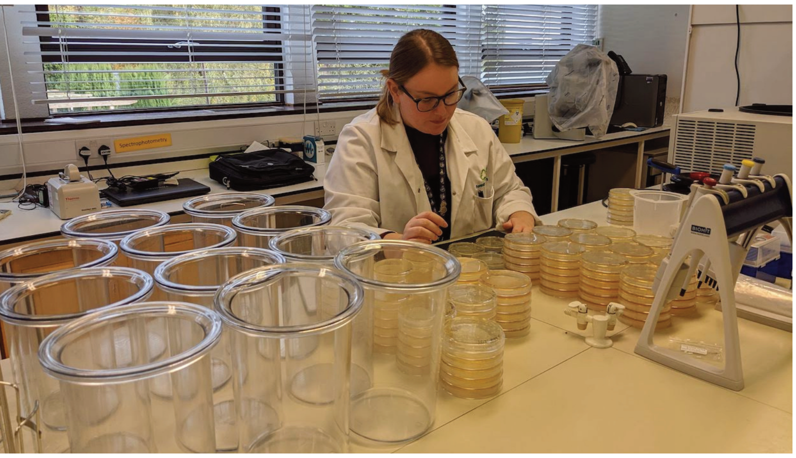


Figure 7. Megan Richardson culturing bacteria isolated from beetle parts in the laboratory with piles of culture dishes. (Photo: Sandra Denman FR)

- Newly emerged unfed beetles tested positive for *R. victoriana* and *B. goodwinii* suggesting that, when the beetles emerge, they are already carrying AOD associated bacteria. It is important to increase the sample size tested for veracity, and to find out if the bacteria are vital.

- The dissected gut samples of beetles fed leaves from the forest, were positive for all four bacterial species (*B. goodwinii*, *G. quercinecans*, *R. victoriana*, *Lonsdalea britanica*) implicated in lesion formation in AOD suggesting that either these bacteria are omnipresent on oak leaves and are consumed by the beetles, or that the bacteria increase in number and detectability once beetles have fed on forest leaves. However, the DNA results do not tell us whether the bacteria remain vital in the beetle gut – this must be demonstrated through culture. Results do suggest that the AOD associated bacteria are present either on forest leaves or in the newly emerged beetle gut (see next point), or both.

- In newly emerged beetles fed clean laboratory leaves very low numbers of *B. goodwinii*, *G. quercinecans* and *R. victoriana* were detected in the beetle gut – yet a further piece of evidence that beetles emerge from bark already carrying a gut microbiota containing the AOD bacteria.

- Beetles fed on laboratory leaves deliberately spiked with the AOD bacteria tested strongly positive in the gut, ovipositor and body of the beetles, again adding evidence to the notion that the beetles can carry the AOD bacteria.

The key research activities for Megan to carry through now are to repeat Michael's work on a large sample size to discover whether his findings are robust, and to confirm whether or not the bacteria in or on the beetles remain vital. If the bacteria do remain vital, the evidence would support the idea that the beetles can carry the bacteria, but further work would be required to show they can transmit the bacteria into host trees.

It also remains to be shown conclusively that the AOD associated bacteria are omnipresent on oak leaves. Over the past few years we have conducted inoculation studies where we place hatching eggs in close contact with bacteria in/on logs and trees to find out whether the hatching larvae can transport the AOD bacteria into the tree. Results to date give good evidence that this occurs, but further experimentation is necessary (Doonan and Denman et al., 2019).

Previously we showed that the activity of the AOD bacteria seemed to change in the presence of the larvae; the bacteria became more active and damaging (Doonan et al., 2020), and lesions on infected trees increased in size (Doonan and Denman et al., 2019). The number of pathogenicity genes increased more than ten-fold when the larvae were present. However, the mechanism by which the larvae cause this increase in bacterial growth and pathogenicity is unknown. Consequently, we are exploring how larvae stimulate bacterial activity, to understand whether small molecules produced by the larvae of *A. biguttatus*, trigger the activity of bacterial pathogenicity genes.

Professor James McDonald (Bangor and Birmingham Universities), Dr Jozsef Vuts (Rothamsted Research) and their team's early career researchers, Dr Marine Cambon and Dr Gareth Thomas, used log surgery to harvest larvae (Figure 8). Elicitor compounds will be extracted from the surface of *A. biguttatus* larvae. These extracted compounds will be identified and their effects on the growth rate as well as pathogenicity gene activity of *B. goodwinii* will be measured. This will tell us if there is a stimulatory effect of the molecules on the bacterial growth and activity.

The Rothamsted Chemical Ecologists in our team (Jozsef Vuts, John Caulfield, Gareth Thomas, Paradip Songara and Godfrey Apangu) are also investigating whether the beetles and larvae are attracted to bacterial odours. If they are attracted to the odours given out by these bacteria it may influence the beetles' preference of which leaves and trees to feed on (Vuts et al., 2016). This

Figure 8. Left to right: Drs. John Caulfield, Michael Crampton, Gareth Thomas and Marine Cambon performing log surgery to retrieve Agrilus biguttatus larvae from oak logs for extraction of larval compounds. (Photo: Sandra Denman FR)

could have serious implications for the spread of AOD, but might also be used as a method of luring beetles into traps.

Management

In the Bac-Stop research project we are also addressing management of the disease. Management of tree pests and disease is a vast topic and while the focus of our discussion here will be on management of AOD, a few general points about management of tree diseases will be made for perspective.

When there is a pest or disease outbreak it is human nature to focus on the specific pest- or disease-causing agent (pathogen) and to want to eradicate it, but as shown in the 'decline disease spiral' model it is evident that the foundation components of AOD are diverse, including not only pests/pathogens, but the environment, the host response and human activities as well. For AOD we aim to research all aspects in the long term, but for now we will discuss a range of possible options that could be applied to decline diseases, looking beyond the reactive response of eradication or control of biotic causal agents to the hope of assisting affected trees to overcome attack and options for preventative management.

Eradication attempts for tree disease often use direct control by removal of infected trees and burning on site or more rarely the use of chemicals (Tainter and Baker, 1996). Cost effectiveness, non-target and environmental impacts are key considerations. Denman et al. (2010) suggested that in cases where only a few trees were affected by AOD, felling and then removing affected areas on tree stems (and burning that material on site) could be considered. The timber is unaffected by AOD and so could still be used commercially. If barked tree stems are sent to a sawmill, affected areas should ideally be treated with an antibacterial agent to lower the risk of spread of bacteria from the weeping patches. Although no experimental data exists for this management option, treating the weeping wound areas with a household antibacterial product prior to moving them to the sawmills could be helpful in reducing the risk of bacterial spread.

Chemical control in forest trees is a very complicated practice and, in reality, has limited safe application. Trees are large, long-lived woody organisms that support whole ecosystems within their leaves, branches, stems and roots. This makes thorough testing of all the impacts of chemical treatments practically impossible and because many of the microorganisms hosted by trees are so vital to their overall health and functional ecology, it would be detrimental to tree health to upset this balance. Thus, caution about the non-target effects of direct control through application of chemicals is often a showstopper in woodlands and forests.

There are a few examples of where fungicide chemical control is used effectively in forestry, for example in nursery settings (Marçais and Desprez-Loustau, 2012). Landscape-scale copper fungicide dispersion by aircraft (Figure 9) is practiced in Australia and New Zealand pine plantations to control the disease *Dothistroma* needle blight (DNB), although this is only one tool in the arsenal used (Bullman et al., 2016). There are several reports of negative impacts of copper on soil microbes and mycorrhizal fungi (Manninen et al., 1998;) and in the UK this practice is not recommended.

Figure 9. Trial of helicopter aerial application mock-up using water and dye to simulate aerial spraying of copper for control of Dothistroma blight as in New Zealand (Photo: David Henderson (Forestry and Land, Scotland), courtesy of Dr Kath Tubby.)

When it comes to applying chemical control of bacterial diseases there is very little evidence to support their use because bacteria develop resistance to chemicals rapidly. Bacterial resistance to copper has already been recorded in shade trees in America (Cooksey, 1990).

There are other avenues of direct control such as engineered microbiomes, which the Future Oak project is investigating, and the use of biocontrol agents (BCAs), endophytes and phages. These all require a huge investment in research and thorough testing before registration can take place, and that would take years if not more than a decade of concentrated research. However, there is good potential to use BCAs in promoting plant health and enhancing resilience in oak and we are currently seeking funding to support this research.

Other components of AOD management include reduction of environmental predisposition stress on trees. Work led by Dr Elena Vanguelova (FR) and team is underway to assess the impacts of drought and nutrient stress as predisposition factors, but much more work will be required to deliver guidelines on soil amelioration. In September 2023 we are hoping, with the help of Woodland Heritage, to fund a student for a PhD study on the effects of different approaches to soil health restoration on oak health and the reduction of AOD. In particular we are interested to find out the effects of amelioration practices on the rhizosphere microbiome. We know that rhizosphere soils around trees affected by AOD are acidic (have a low pH) (Pinho et al., 2020) and that the soil pH also affects the soil and tree root microorganisms and the functions they perform. For example, ammonium (NH_4^+) and nitrate (NO_3^-) are the only forms of nitrogen (N) that are usable to most plants, and nitrogen (N) is one of the key plant nutrients for growth, but plants are unable to use it in that form. Nitrogen needs to be converted into plant-usable forms and ammonia-oxidizing bacteria (AOB) and ammonia-oxidizing archaea (AOA) are the main organisms that do this. Healthy oaks have a high abundance of AOB correlating to less acidic conditions (Scarlett et al., 2021). If soil pH drops and soils become acidic, plant-available nutrients also become limited. Much more research is required on soil relationships and AOD occurrence but one practical control measure that could emerge from more research is the amelioration of soil acidification by balancing C:N ratios, but it is important to find out the best method of doing this. In previous work we undertook on restoring the soil condition of the Major Oak in the Sherwood forest, where mulching the soil around the tree with bark pieces had been applied, we found that this mulching activity using un-composted bark as a layer treatment actually stressed the tree by preventing water from reaching the tree roots and depleted soil nutrients, which were probably taken up by microorganisms that were in the process of decomposing the bark and therefore not available for tree growth (Vanguelova et al., 2016).

Silvicultural aspects such the use of thinning to reduce stress is always advocated in principle but prescriptive advice is difficult as there are so many other factors that influence the tree's environment and management ambitions, so thinning practices are often best left to forest managers' discretion.

> "When it comes to applying chemical control of bacterial diseases there is very little evidence to support their use."

In partnership with Kew (Professor Richard Buggs and Dr Romula Carleial) entire genomes of AOD and unaffected oak are being sequenced and analysed in the search for inherent resistance properties. We are still some way off the final results of this study but potentially there could be a way of selecting tree genotypes that are disease tolerant.

Additionally, in the Bac-Stop project we are gathering social evidence to uncover stakeholders' knowledge and experiences, values, attitudes and motivations to act in relation to oak health and its management, through the work of Liz O'Brien (FR) and her team. This information will help us better communicate with and assist our stakeholders in managing AOD.

Communication of research

Research on AOD in the UK has been undertaken since 2008. We have worked extremely hard on making our research results available to as wide an audience as possible. To date 60 peer reviewed articles and 33 popular articles have been published. Several times a year we tweet our activities on the FR and BPD websites, and we have participated in radio and television interviews. We have an oak enthusiast group that we regularly update on research results by giving members plain English summaries of the latest findings, and we frequently present our results at seminars, forest health and charity-organised events. If you would like more information or if you would like to be added to the mailing list, please contact Sally Simpson (Woodland Heritage) Sally.Simpson@forestresearch.gov.uk.

Closing comment and acknowledgements

There is no doubt that AOD is a highly complex disease, and this has necessitated the involvement of colleagues from a range of scientific disciplines resulting in a truly multidisciplinary, integrated research effort. This approach has yielded great returns in diagnosis of the disease and its drivers, and has already provided stakeholders with much information and management guidance. Further research will continue to provide information, and as science progresses, new disease management tools will be tested and should become available.

As ever, our work could not progress without the incredible support we receive from funders (specifically Defra, the BBSRC, NERC, the Scottish Government and the Forestry Commission), charities (especially Woodland Heritage, but many others including the National Trust, Woodland Trust, Monument, Rufford and the John Paul Getty Trust), landowners, forest managers, FR's Technical Support Unit and Sally Simpson our project support officer. Furthermore, the interest, dedication and commitment from our researchers is truly inspiring. We thank you one and all for your generosity, enthusiasm and commitment, together we are making things better for trees.

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