ActionOak
PROTECTING OUR OAK TREES

Action Oak Report

2022-23

Contents

Front cover image © Georgina Colman

Foreword

by Lord Benyon, Minister for Biosecurity, Marine and Rural Affairs

Image © Georgina Colman

Oaks are the UK's most iconic trees, and national symbol of strength and power. I cannot overstate the contribution our Oaks make to our landscapes, biodiversity, culture and the economy.

These trees are under considerable and growing threat from pests, diseases, human intervention and the nature and climate emergency. We need to act now to protect them for the future.

The government's recently published Plant Biosecurity Strategy for Great Britain highlights ninety pests and diseases that can attack Oak. Some of them, including Oak Processionary Moth, Acute Oak Decline, honey fungus and powdery mildews are already present in the UK and weakening or killing our magnificent Oaks. Others, like *Xylella fastidiosa* a bacterial disease spreading across Europe, pose a future threat and are being monitored closely through our international networks.

There is a vital need for more research to inform our action. The pioneering progress Action Oak has already made, supporting thirteen new PhDs and bringing together over thirty partners from across scientific, charitable and government sectors to help protect our Oaks, is recognised in the new biosecurity strategy. Under the umbrella of the Action Oak Partnership, researchers across the UK are monitoring and investigating the health and condition of our nation's Oaks. The data they gather is shared with tree owners and practitioners to inform management activity on the ground, devise real world solutions and optimise resistance to the threats these iconic trees face.

This Annual Report plays an important role in sharing the knowledge generated by those leading researchers. I encourage you all to read it and further support the important work of Action Oak.

Introduction

by Geraint Richards, Chair of Action Oak

As I reflect on the work of Action Oak over the past twelve months, perhaps the most significant element has been the ability, 'post-Covid', to return to occasional in-person meetings and events. A high-profile dinner was kindly hosted by the City of London at the Guildhall in May 2022, a well-attended Partner Event was held at the Royal Botanic Gardens Kew in June 2022 and then the Steering Committee and KEMP Sub-Committee enjoyed an excellent meeting at the Yorkshire Arboretum in November 2022. The generous support for Action Oak, by these organisations and many others, shows how seriously the health of our native oak trees needs to be taken and hence how vital the work of Action Oak is. There is so much more that needs to be done in the short-term, for the long-term, and hence I am delighted that, due to Defra funding, we are in the process of recruiting a Programme Leader who will work with us all to raise the profile of Action Oak and hence increase the outputs.

These are exciting times for Action Oak and I want to thank you for your continued support and I look forward to working with you all over the next twelve months.

Image © Georgina Colman

Monitoring the health of Oak trees across Great Britain

by Nathan Brown, Forest Research

The health of trees can often be seen in their crowns. The density of leaves on a tree's branches directly influences the amount of energy it can capture through photosynthesis, which in turn influences tree growth. By monitoring crown condition, we gain an insight into the underlying health status of Oak and the potential impact of environmental factors on tree condition. The Forest Condition Survey ran from 1987 through to 2007, documenting the health of five tree species. Following the Action Oak Knowledge Review funding was provided by the Forestry Commission to revisit 85 Oak plots. Since 2019 Forest Research staff (TSU) have located, remarked and assessed trees in these plots using the original methodology as well as the extended measurements needed to phonotype Oak trees affected by decline.

Collaborative work between researchers from Aberystwyth University and Forest Research, funded by Woodland Heritage and Defra, has highlighted the importance of crown condition assessment in determining the severity of decline in Oak trees. This work was based on a comprehensive set of visual assessments that include aspects of tree size, crown condition and the presence of disease-causing organisms. This allows the trees size and relative stature within the stand to be calculated so that long term health is documented in addition to current crown health.

In summer 2022 condition surveys were conducted at 83 condition survey sites were assessed using the full phenotyping protocol, and quality control checks were conducted at 10 of the sites. Preliminary results are shown in figure 1, which shows the changes in average crown transparency over time. This is a crude measure, but it gives a quick overview of long-term trends. Data from the original survey is shown in dark blue and a model prediction based on this data is shown as a thin straight line. Data from the last three years is shown in green and a model based on all data 1989 to present is shown as a thin green line. Both models show that crown transparency is increasing over time (canopy health is worsening). However, it is clear that the model based on only 1989-2007 data overpredicts the rate of decline and this is corrected by including the most recent three years of data.

In July 2023 we plan to hold training workshops to describe the processes involved in condition assessment so that tree owners, managers, and volunteer groups can assess the health of trees. As part of the Bac-Stop project (led by Forest Research) methods and training materials have been developed and we are currently planning events where we can demonstrate the methods. Thank you to all the volunteers who gave their time to help our project so far.

We will shortly be advertising the workshops for this summer, so if you are interested, please look out for our adverts and do contact me if you have any questions: **nathan.brown@forestresearch.gov.uk**. We will ask volunteers to attend a one-hour webinar, which will be followed by a half-day training workshop in the field. This should provide all the information and tools

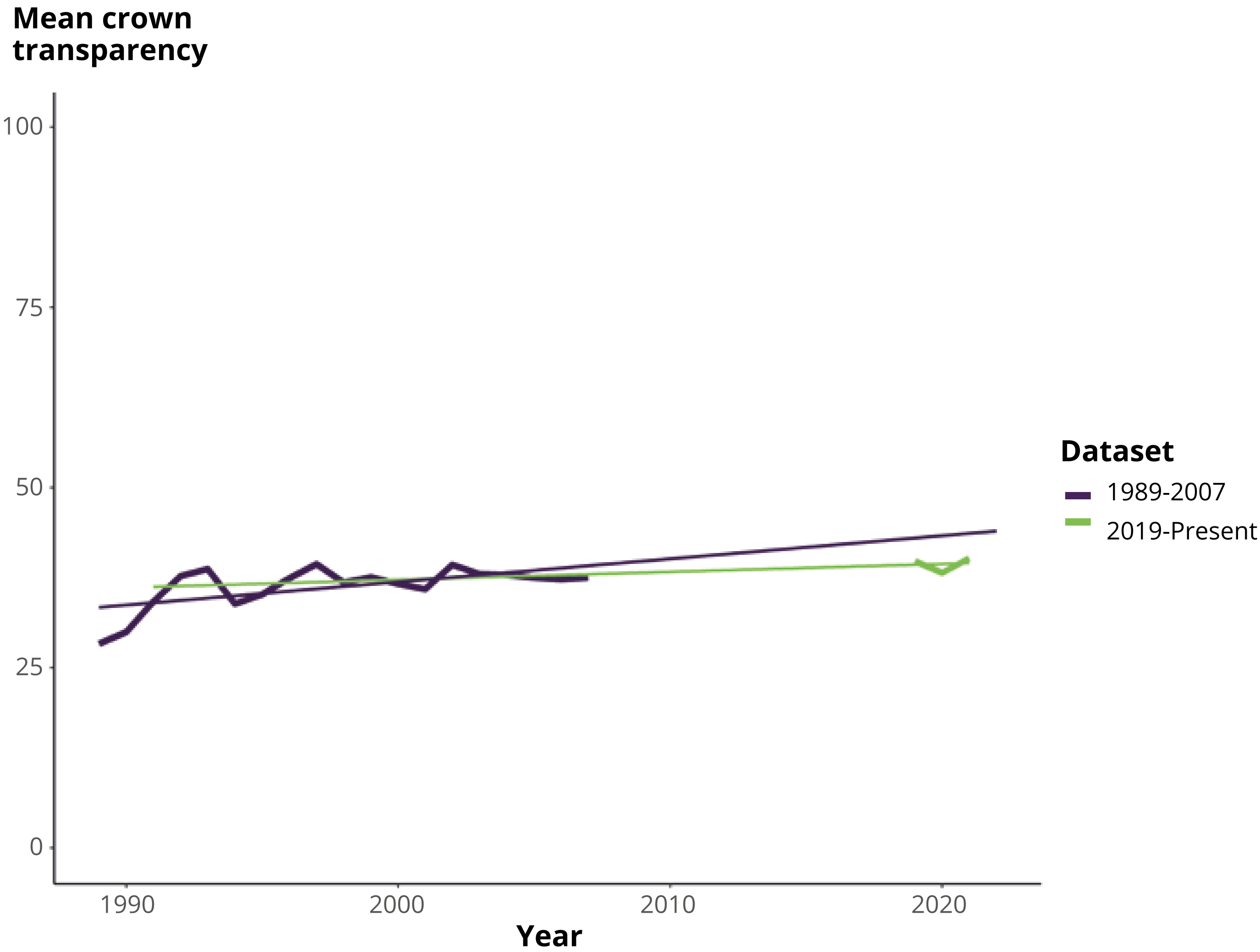


Figure 1: Changes in mean crown transparency over time

Figure 2: A training day in Richmond park

required for further monitoring and enable individuals to observe the trees that are most significant to them and provide data that can be integrated with an established monitoring program. One of our key aims is to instil an interest in observing change in the natural environment, as well as enabling tree health to be documented in a consistent way.

forestresearch.gov.uk
bacterialplantdiseases.uk

Annual report on AOD research – March 2023

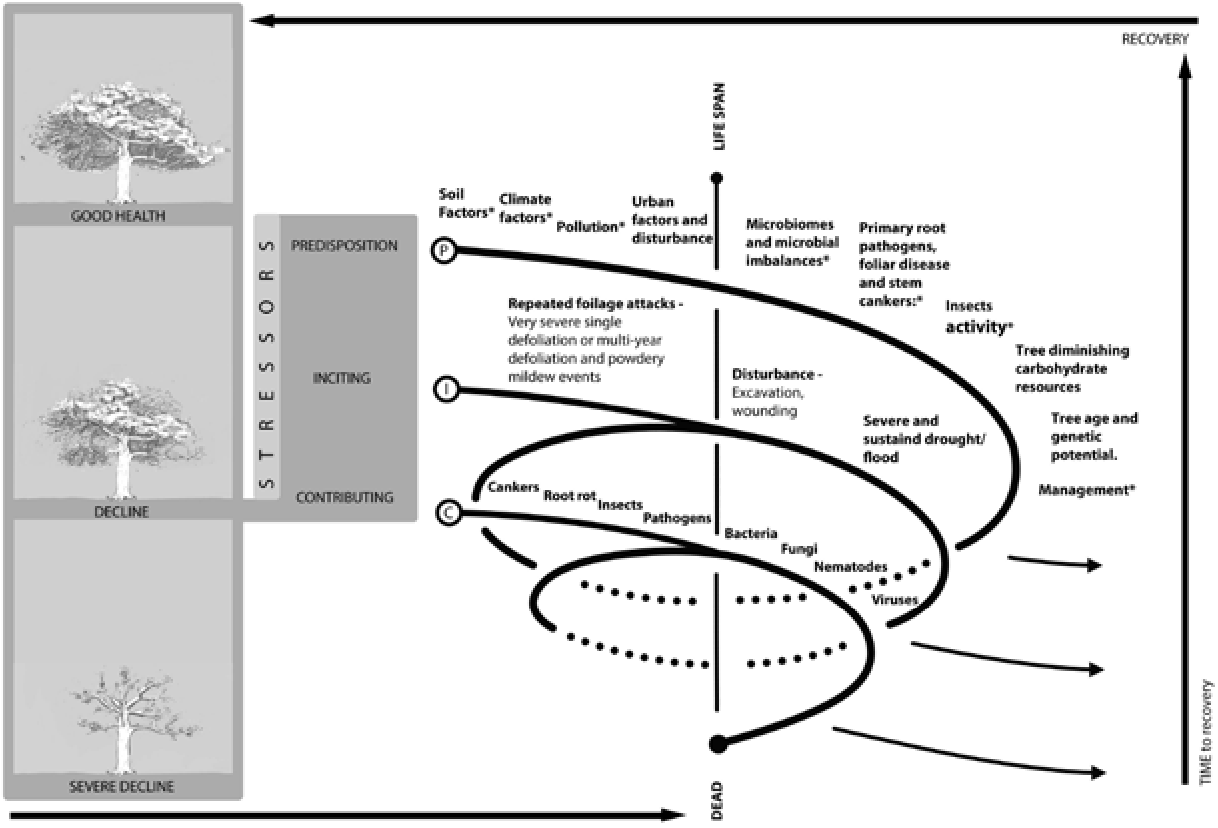
by Sandra Denman, Forest Research

In 2020 the Bac-Stop team was awarded a UKRI grant, financed by Defra, BBSRC, NERC and the Scottish Government and supported by Action Oak. The aim of the research offered was to investigate particular environmental factors that weaken trees and make them more vulnerable to Acute Oak Decline (AOD), and to investigate if the native Oak jewel beetle (*Agrilus biguttatus*) is essential to the development of AOD. We were also tasked with testing other forest tree species for the presence of the AOD associated bacteria and their susceptibility to these microbes, and finally, to finding out the attitudes and values of stakeholders such as landowners and forest managers, to Oak trees and management of AOD, so that we can ensure best communication with stakeholders and assist with management. AOD is a destructive, emerging disease in the UK. It is a complex disease caused by environmental predisposition drivers that weaken trees allowing insects and micro-organisms to colonise trees in a damaging way. This is a special type of disease called a Decline disease that involves a great number of complex interactions.

The concept of Decline diseases was first developed in the United States by forest pathologists Wayne Sinclair (1967) and Paul Manion (1981), who developed a model called the Decline Disease Spiral Model (Manion 1981) to help explain the factors involved. Last year under the Bac-Stop project we updated the model (Figure 1) and wrote a textbook chapter (Temperate Oak Declines: Biotic and abiotic predisposition drivers, S Denman, N Brown, E Vanguelova, B Crampton, Forest Microbiology Volume 2: Forest Tree Health, 239-263 (2) 2022), explaining some of the challenges in identifying, diagnosing and researching Decline diseases.

Over the past 15 years our research on AOD has been guided by the Decline Disease Spiral model. To date we have identified the distribution of AOD in the UK as well as key predisposition factors correlating with environmental factors 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).

We are now in the process of testing some of these drivers (drought) to obtain empirical evidence and data that will allow us to model the risks of AOD outbreaks and the potential for its increasing occurrence, especially in changing climates. We set up a field trial where we constructed rain exclusion shelters around the stems of Oak trees (Figure 2) and measured the effects of excluding water (drought) on the physiology and function of the trees as well as on the microbiome and AOD severity. We are also testing the effect of ringbarking trees as a proxy for nutrient stress and the corresponding effects of this on the microbiome and disease severity. The trial has been running for nearly three years and has one more summer season to go but provisional results show that the rain exclusion shelters have worked well in creating drought stress conditions, and that



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*, *Gymnopus, 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 1: The updated Decline disease spiral model showing predisposition drivers and the process of Decline disease development

Figure 2: Rain exclusion shelter constructed around stems of Oak trees to simulate drought under experimental conditions

Figure 3: Agrilus biguttatus adult female beetle

Figure 4: Megan Richardson culturing bacteria from beetles

trees in this treatment already show impacts of drought, for example having reduced leaf size. Results of the trial effects on the health of the trees should be available for the newsletter next year.

Our work in the Bac-Stop project investigates whether or not *A. biguttatus* (Figure 3) is essential to the development of the disease. Previously we stated that the potential role of *A. biguttatus* in AOD is controversial and difficult to prove definitively, partly because it is extremely difficult to get these elusive beetles in hand. However, we have been lucky in acquiring a good supply of beetles over the last season in particular, thanks to generous landowners and managers who have supplied us with bark slabs that contained the beetles.

We want to find out whether the beetles are carrying or are able to carry bacteria and whether the interaction of the beetle larvae and AOD associated bacteria, specifically *Brenneria goodwinii*, which is the most damaging bacterial species in the AOD pathobiome, is essential to causing the bacterial degradation of live tree tissues. Four treatments were set up: (a) Newly emerged unfed beetles; (b) beetles fed on 'clean' Oak leaves grown from seed in incubators; (c) Spiking the clean leaves into an AOD bacterial solution; and (d) feeding beetles on natural forest leaves. The whole beetles as well as the dissected gut and ovipositor, together with beetle eggs, larvae and frass were tested for the presence of the AOD associated bacteria using culture methods and DNA detection. Working on the Bac-Stop project, this work was initially pioneered in 2020 by Dr Michael Crampton but has been taken over by Megan Richardson who joined us in July last year and has already dissected 180 beetles and cultured several thousand bacterial isolates! (Figure 4). Preliminary results (Crampton 2020) showed that:

1. Using DNA techniques only, we discovered that newly emerged unfed

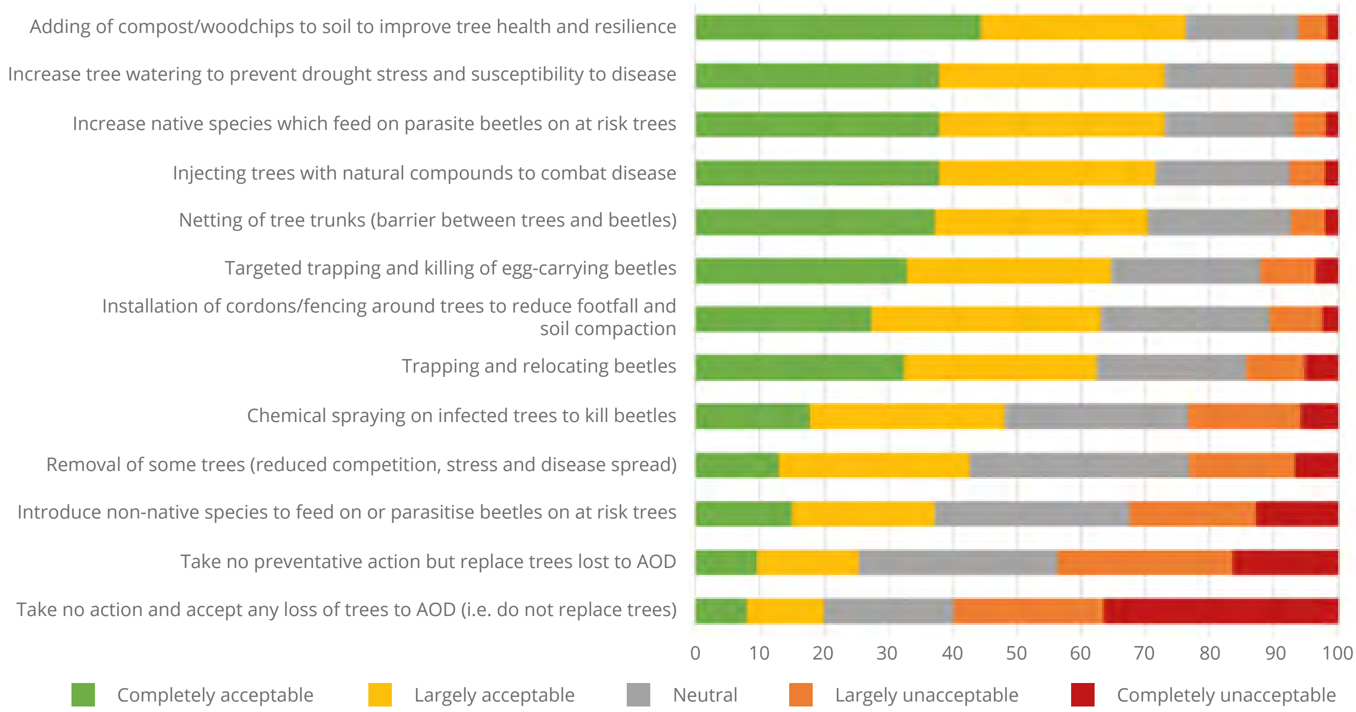


Figure 5: Acceptability of different methods to protect Oaks

beetles tested positive for 2 of the 4 AOD associated bacteria *R. victoriana* and *B. goodwinii*. Although only a small sample was tested these preliminary results suggest that when the beetles emerge, they are already carrying AOD associated bacteria, but we need to culture the bacteria to see if they are vital or not.

2. 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. This is a further piece of evidence that beetles emerge from bark already carrying a gut microbiota containing the AOD associated bacteria, but again we do not know whether the bacteria were living or not.

3. It was also evident that in the dissected gut samples of beetles fed leaves from the forest, that all four bacterial species (*B. goodwinii; G. quercinecans; R. victoriana; Lonsdalea britanica*) were present, pointing to the possibility that the bacteria are omnipresent on Oak leaves in forests and are consumed by the beetles.

4. In beetles that were fed on laboratory leaves deliberately spiked with the AOD bacteria, tests were strongly positive for these bacteria in the gut, ovipositor and body of the beetles. This suggests that the beetles can carry and consume the AOD associated bacteria, but we still need to verify these results with more replication and to confirm whether these bacteria are still vital after passaging the beetles.

Although we have made good progress this work was developed on a very small sample and so there is still a lot to be done. It also remains to be shown conclusively that the AOD associated bacteria are omnipresent on Oak leaves and whether by eating the Oak leaves with bacteria in/on them the adult beetles pick up the bacteria. Alternately, the bacteria exist harmlessly in the stems of Oak trees. We will try to simulate this in experiments next year to see if bacteria make contact with the larvae of *Agrilus* in this way. Over the past few years we have conducted inoculation studies where we place hatching eggs in close contact with bacteria in / on log 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.

In the Bac-Stop project specific investigations into the interaction of the beetles and the bacteria with the aim of finding out whether larval compounds can induce pathogenic gene activity in the bacteria are underway. Elicitor compounds will be extracted from the surface of *A. biguttatus* larvae, identified and their effects on the growth rate as well as pathogenicity gene activity of *B. goodwinii* will be measured.

To assist management implementation, we engaged with the public, and carried out a survey of 6,000 UK residents to explore how people value Oak, what their awareness of AOD was and to ask whether people think the different methods to protect Oak might be acceptable or not (Figure 5). Some of the management approaches listed are hypothetical or not yet tested. In general, we found that people are most likely to oppose approaches if they are perceived as being ineffective, unsafe or result in broad impacts, for example, if they pose threats to other species. In the specific case of AOD, people tend to prefer measures to ensure trees are in good health and thus resilient to diseases therefore focusing on prevention rather than cure.

We are also examining whether bacteria, in particular *Brenneria* spp., play a role in stem bleeding diseases of tree species other than Oak, and whether the AOD pathobiome bacteria can be found in other tree species.

This work is carried out in Bac-Stop WP4. To date, we have obtained 119 swabs from 19 different tree species showing stem bleeding symptoms, from which we isolated 137 bacteria. After identifying these bacteria, we found some of them were new to science and so we have just completed characterising four novel species. These novel species include *Brenneria tiliae* (from Lime trees), and *Winslowiella arboricola* from Lime trees (*Tilia* x *europaea*) and London Plane (*Platanus* x *acerifolia*). Tests are underway to determine whether these novel bacterial species are damaging to trees. In addition, we isolated AOD-associated bacteria specifically *Brenneria goodwinii* (the most damaging in the AOD pathobiome) from non-Oak hosts. We have performed pathogenicity trials in which these isolated AOD bacteria were inoculated onto Oak and four non-Oak hosts. The trial has been harvested and we are currently analysing the results.

To date we have published more than 60 peer-reviewed scientific articles and 33 popular articles. We have initiated an Oak Enthusiast group and provide half page summaries of our new findings for this membership several times a year.

Please contact **Sally Simpson** (**sally.simpson@forestresearch.gov.uk**) if you wish to join this group membership. We also hold an annual stakeholder meeting where we present our results, and we give multiple online and in person seminars as well as relating our research in real time on the FR and Bac-Stop Twitterfeeds.

We thank Action Oak and our funders for their support, as well as the many landowners, charities, policy advisors and the public for their interest, help and encouragement. We love our work and are completely committed to doing our best to promote the health and resilience of native British Oaks.

Improving the understanding of Acute Oak Decline: from disease development to biological control

by Emily Grace, Vanja Milenkovic, Diana Vinchira-Villarraga, Mojgan Rabiey, and Robert W Jackson, University of Birmingham

Acute Oak Decline is a severe disease that affects UK native Oak species (*Quercus robur* and *Quercus petraea*). Several attempts have been made during the last decade to characterise the disease and the pathogens responsible for it. Further efforts have been made to understand how the disease develops, how the plant reacts to the pathogens infection to overcome the disease, how different environmental factors, such as soil health, affect the disease outcome and which control methods could be developed to reduce the severity and incidence of AOD in Britain. To contribute to the study of this complex disease, our research group has established three projects that aim to (i) describe how Oak trees respond to the infection with the three primary pathogens related to AOD (*Brenneria goodwinii* (Bg), *Gibbsiella quercinecans* (Gq), and *Rahnella victoriana* (Rv)) under controlled conditions, (ii) to understand how the pathogens gene expression and metabolism change during the interaction with its host, (iii) to evaluate if (and how) the soil can influence the tree health and disease development and (iv) to evaluate if bacteriophages can be used as a control strategy to treat and/or prevent AOD.

Metabolic profiling of healthy mature Oak bark was carried out to delve into the first aim. The main chemical groups of metabolites extracted from Oak bark were identified using *in silico* tools. More than 4000 features were detected, from which 3% were annotated based on their MS/MS data 37%, whereas 37% were classified at the superclass level, being organic acids and lipids (and lipid-like molecules) the most abundant classes. The fact that 63% of the detected features remained unknown highlights the chemical diversity of this tree tissue and how more efforts need to be made to overcome the boundaries of known plant metabolites. This dataset will be used as a baseline for future *in vivo* experiments that seek to compare how the tree's metabolome changes once it is challenged with the pathogens.

An *in vitro* interaction using Oak shoots inoculated with Bg, Gq, Rv or a co-culture of the three strains was performed to address the second aim. The bacterial growth, metabolites, and RNA were obtained and evaluated from these samples. So far, the data have shown that the strains have a differential growth when they are inoculated individually compared to when they are co-inoculated. Similarly, the metabolism of the pathogens changes when they interact with each other and in the presence of the host. The nature of these changes is currently being evaluated to select which biological markers (metabolites) can be further followed during *in vivo* Oak-pathogen interaction under controlled conditions.

A three-year field experiment is being carried out as part of the third aim. In the experiment, 24 Oaks from two woodlands are being monitored. Half of the Oaks show symptoms of AOD, and the other half are not showing symptoms so far. As these trees are being used to evaluate if soil can impact tree health, half of the symptomatic and half of the non-symptomatic trees have been treated with mulching. Soil, leaf, and twig samples have been collected from all trees involved in this trial during May, September, and November, alongside phenotypic measures related to tree health status. Both the soil and leaf samples will be used for microbiome analyses, and the leaf and twig samples will be used for metabolome analyses. From the phenotypic characterisation of the trees, some differences between diseased and healthy trees have been observed. However, no effect of the treatment has been detected so far. We will continue monitoring the trees for a further two years to observe the outcome of the applied treatment and describe the changes in the metabolism and microbiome in the evaluated trees.

Finally, to assess if bacteriophages (viruses that infect and kill bacteria) can be used for AOD control, a collection of phages that infect key bacteria species within the Acute Oak Decline pathosystem (*Brenneria goodwinii* and *Gibbsiella quercinecans*) was obtained from naturally infected trees. Specifically, two sets of phages that infect these respective species have been isolated from diseased lesion tissue of Oak trees from woodlands in the Midlands, UK. They have been characterised to assess their suitability as biocontrol agents. Thus far, the phages have been found to effectively reduce their host populations *in vitro* and are likely safe as they do not infect non-target bacteria species. Furthermore, this project will track phage populations in diseased and healthy trees in an Oak woodland over different seasons to determine if they influence Oak health and AOD disease progression. Future work will include analysis of phage dynamics when placed in a system containing both bacteria and testing the impact of phages in preventing lesion formation *in planta.*

birmingham.ac.uk/research/bifor/index.aspx

Looking across the site to the Wrekin

Action Oak update – Royal Botanic Gardens, Kew

by Louise Gathercole, RBG Kew, QMUL & Centre for Forest Protection

Last year we reported on a paper published by one of our Action Oak supported PhD students Gabriele Nocchi. Gabriele has now successfully completed his PhD and is now working as an Evolutionary and Ecological Postdoctoral Associate at the University of Calgary.

I am the second PhD student at Kew funded with support from Action Oak, and at the end of last year I published a paper looking at the genetic evidence for Acute Oak Decline bacteria on the leaves of Oak trees. This used the whole genome re-sequenced data from our first set of sequenced samples, which was funded by Action Oak. My analysis found that there is genetic evidence for the existence of AOD-associated bacteria on the leaves of the Oak trees and there are no differences between healthy and affected trees in our samples. The paper is open access and can be found here: **mdpi.com/1999-4907/12/12/1683**

The bar charts below show the proportion of healthy, symptomatic and remission trees where we found bacterial DNA on the leaves that mapped to the acute oak decline bacteria.

I am now looking at the distribution of the two native species of Oak, their hybrids and genetic evidence for adaptation to the environment in a second set of whole genome re-sequenced samples, which was funded by Defra.

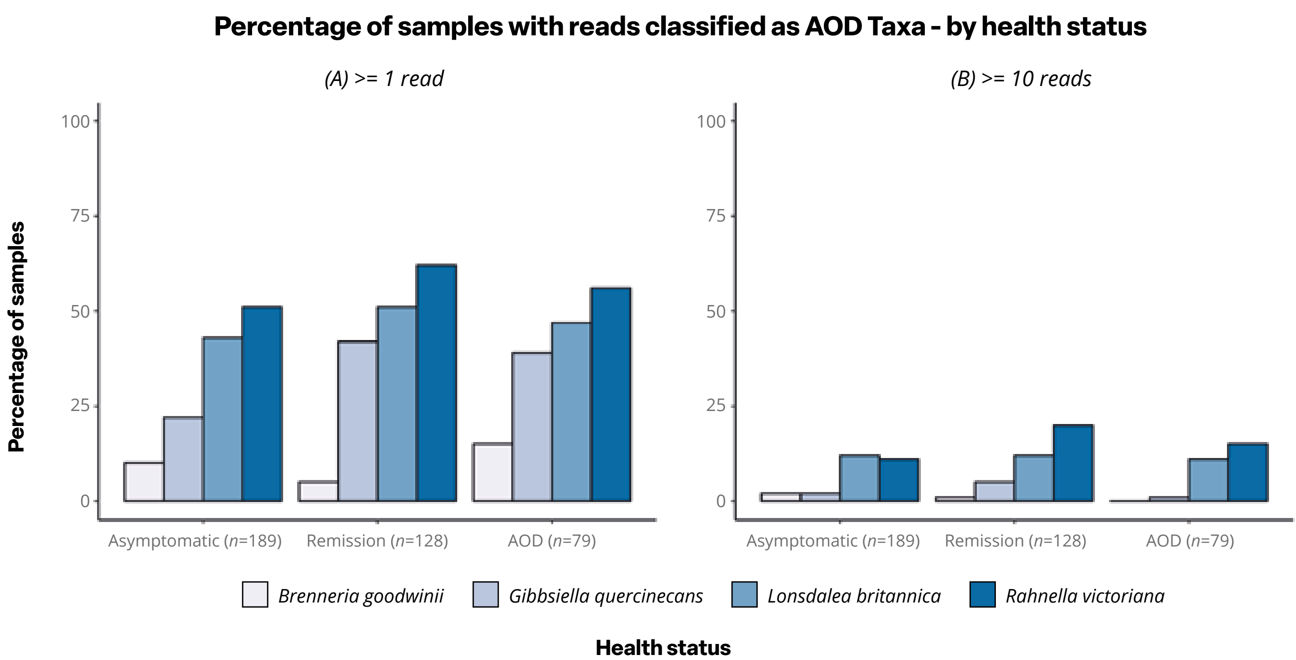


Image © Louise Gathercole

One of our parkland sampling sites where there are trees with Acute Oak Decline symptoms alongside healthy trees

I have also begun working as the Coordinator for the Centre for Forest Protection (CFP), which is an initiative that continues the collaboration between Kew and Forest Research and has a vision and mission closely aligned with that of Action Oak. The mission of the CFP is to enhance the resilience of the UK's forests, woodlands and trees and protect them from environmental and socioeconomic threats, through the provision of evidence, interdisciplinary research, expert advice, and training.

The Centre for Forest Protection has a further connection with Action Oak. Our work on oak at Kew is now feeding in to a CFP project to build a large panel of sequenced oak genomes. These will be used to carry out a genome wide association study (GWAS) on the susceptibility of Oak to Acute Oak Decline and to look at the genetic contribution to other traits in Oak trees. This work is being carried out by Dr. Rômulo Carleial, a Postdoctoral Research Associate with the Centre for Forest Protection at Kew. The idea of the AOD GWAS is to find out whether there is a genetic association with susceptibility to the disease, and if so, to identify genes that may be associated with resistance. Preliminary results show some promising candidate genes and the team will be investigating whether these genes could be involved in insect, bacterial or stress responses that impact on the ability of the tree to resist the contributors to Acute Oak Decline.

forestprotection.uk

Investigating the Oak root microbiome, two years later

Dan Maddock, University of West England

Back in 2021, I wrote an article for Action Oak a year into my PhD project which was to examine the role of the rhizosphere (the region of soil surrounding the root proposed by Lorenz Hiltner back in 1904) in the development of AOD. Two years later and I've finished and submitted my thesis and can happily say I've made some exciting discoveries concerning my three aims.

My first aim was to use synthetic long-read 16S rRNA sequencing to identify significant differences between the community composition of bacteria in the Oak rhizosphere at the species level. This proved to be a fascinating avenue to pursue, which revealed that healthy Oak supports more diverse bacterial communities in the soil when compared to AOD symptomatic Oak. However, this difference was only seen in parkland samples and not in the woodland, implying the effect depended on the soil environment. The impact of these differences is hard to quantify, though large numbers of plant growth-promoting bacteria were significantly and, in some cases, exclusively associated with the healthy Oak rhizosphere, including nitrogen fixing, bio-control and abiotic stress relieving bacteria.

My second aim was to identify a point of origin for the four bacteria associated with AOD using selective enrichment of Oak-related samples and molecular analysis for their identification. This work was ongoing through much of my project. After processing a large number of rhizosphere, leaf and acorn samples with minor adjustments to the enrichment method we

AOD afflicted Oak

A rhizosphere sample taken from the Oak on the left

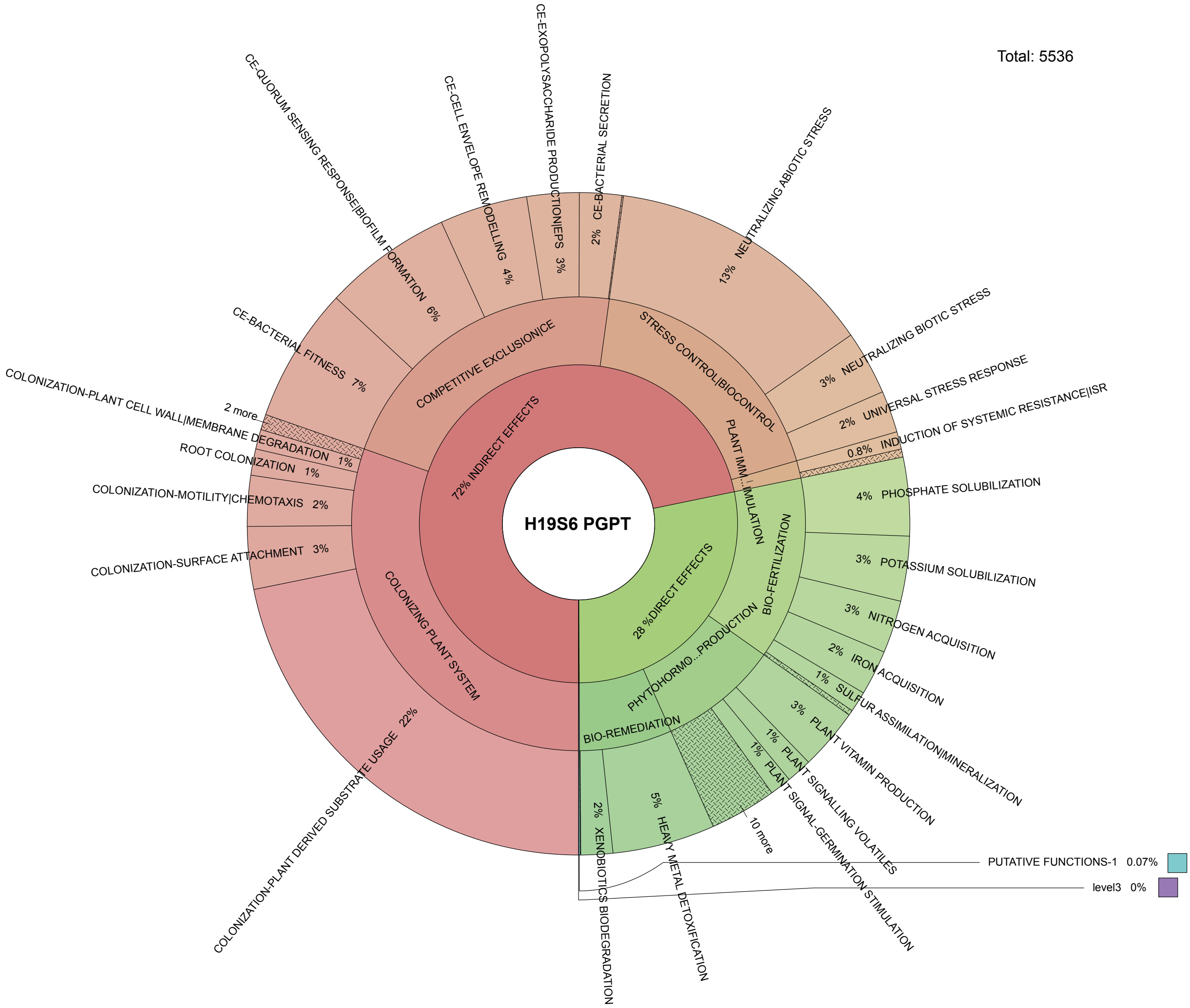
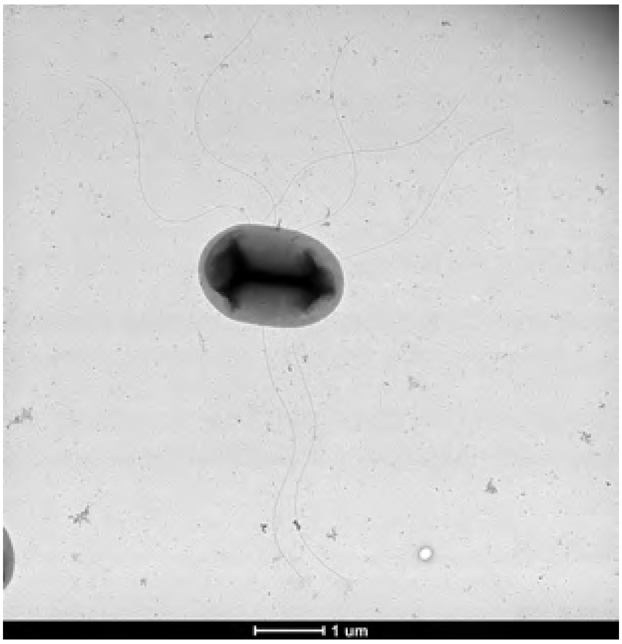


Figure 2: The Krona plot of the plant growth-promoting traits identified from Leclercia tamurae H6S3T

have only begun to scratch the surface of where the AOD bacteria originate from. The overall findings imply that both *Gibbsiella quercinecans* and *Rahnella victoriana* are widely distributed within the environment, and are consistently isolated from soil, leaves and acorns. Meanwhile, *Brenneria goodwinii* and *Lonsdalea britannica* were not isolated from soil, despite being recoverable from lab-inoculated soil samples for six weeks. Instead, they only appeared to be recovered from inside the tissue of acorns from both healthy and diseased trees, indicating they might be seed-inherited endophytes. To come to a conclusive statement, this work would need to be continued on a larger scale over several different sites, however it is a step in the right direction.

My final aim was to classify as many novel species of bacteria isolated from the roots of Oak as I could identify. The rhizosphere of Oak contains such large numbers of undiscovered bacteria that this wasn't possible. Instead, I took a subset of the



The transmission electron microscope image

isolates that we identified during my PhD and chose to classify them based on their relation to other rare or interesting bacteria. In the end, we published three papers on four different genera of bacteria, two of which were novel. Overall, I classified eight new species of bacteria, and through comparison of their whole genomes to different databases highlighted the potential of five to be novel pathogens and the remaining three to play roles in the environment as plant growth-promoters. An example of the plant growth-promoting novel species *Leclercia tamurae* is shown in Figure 2.

I'm incredibly proud of the work I've achieved throughout my PhD, and I hope the findings highlight the potential that investigation of the Oak rhizosphere offers us. From potential sources of pathogens and useful plant growth-promoting bacteria to playing a role in the decline disease spiral, this untapped area deserves and requires further work to be well understood. I want to thank UWE and Woodland Heritage for the opportunity to contribute to such exciting and important research. I'm happy to report that this is not the end of my journey in Oak research but that I will continue to work with my supervisor, Dr Carrie Brady, on a short post-doc where we will be performing whole genome sequencing on many different *B. goodwinii* strains from multiple countries and sites. This project aims to determine if we can identify an evolutionary difference based on the time and location these isolates were collected from. Understanding this evolutionary distribution will help us understand if their pathogenic ability has increased, relating to their increasing occurrence. The larger aim will be to start identifying conserved features with these pathogens and other woody pathogens to see if it is possible to identify what allows bacteria to cause disease on trees. Can we use these features to predict host range and the ability of new pathogens to cause disease on trees in the future? I hope that through this new project I can continue to contribute to the field of Oak research and help protect our flagship native tree!

FUTURE OAK project research update

by James McDonald et al., Birmingham University, Bangor University

The leaf, stem and root tissues of plants are densely colonised by trillions of micro-organisms (e.g. bacteria and fungi) that have many beneficial properties for the plant, including the promotion of growth, provision of essential nutrients, stimulation of plant immune systems, increasing stress tolerance and suppression of pathogens. The collection of micro-organisms found in a particular part of a plant and the activities and interactions they have with each other, and their plant host, is called the 'microbiome'. Oak trees are increasingly exposed to threats from environmental stressors such as drought, and attack by pests and diseases, which can weaken host health and affect the composition and function of the microbiome. However, we have a very limited understanding of the composition and function of the microbiome of Oak trees, and how they protect Oak health or are impacted by the stressors that Oak trees experience.

The FUTURE OAK project aims to address this knowledge gap by characterising the Oak microbiome and associated chemical profiles (the 'metabolome') across the UK landscape. In summer 2021, we collected root, stem and leaf tissue from 350 Oak trees at 30 sites across England, Scotland and Wales. The sampled sites and trees were chosen to reflect a range of Oak health categories, in particularly relating to the presence and severity of Acute Oak Decline (AOD), an emerging disease of Oak threatening woodlands in the UK. These samples are currently being analysed to study the Oak microbiome and metabolome. Our project also has a significant social science component that explores how UK forest managers' manage Oak woodlands, including an assessment of their perceptions of, and ideas about, the Oak microbiome.

Oak health and the microbiome across the UK landscape

To understand the relationship between the environment, the microbiome, and Oak health, the Bangor University team have been characterising the microbiome of the sampled Oak trees using a DNA-sequencing approach that can identify all microbes within a sample, allowing deep understanding of the composition and function of the microbiome. We have nearly finished the initial data gathering phase of this work, and preliminary analyses are already beginning to provide some core insights into the taxonomic composition of the Oak microbiome, as well as its structure. So far, we have shown that among the fungal species, the microbiome of the leaves, stems and roots are highly distinct, and most fungal species found are unique to specific

Image © Dan Struthers

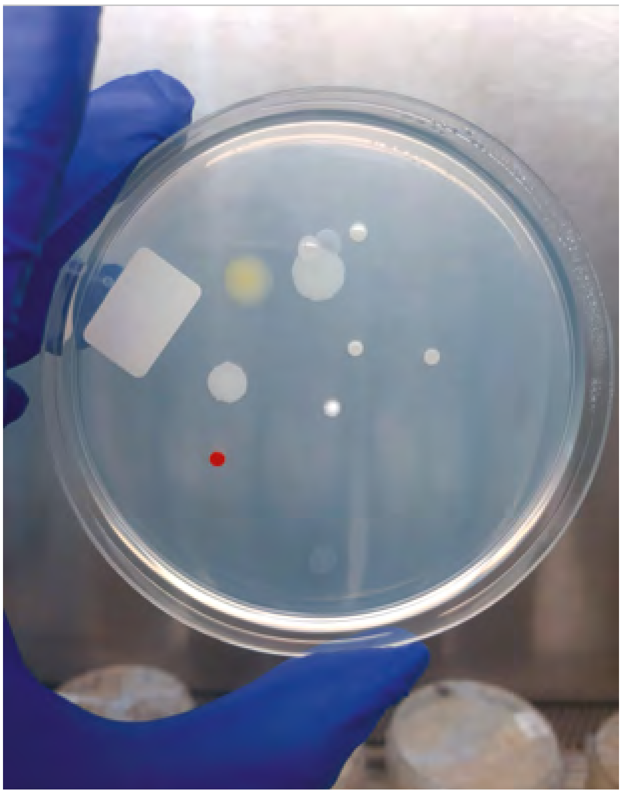
Coed Aber

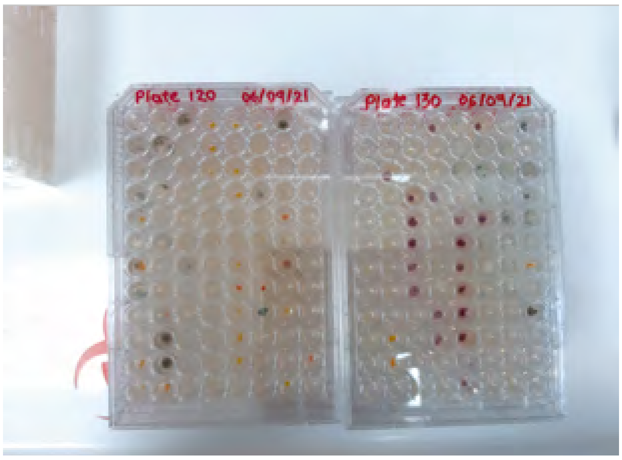
tissue types. Future analysis will explore the relationship between the microbiome and environmental factors such as climate and soil, and seek to identify microbial species that are strongly associated with Oak health for further study.

The Aberystwyth University team has performed metabolite extraction and metabolome fingerprinting analysis to measure the chemical composition of the Oak samples. The metabolome is the collection of small-molecule chemicals (metabolites) that can be found in a biological sample and can be an indicator of health status. They are currently analysing this landscape-scale data set to understand how environmental factors such as climate, soil and location influence the Oak metabolome. Phenotypic decline indexes have been generated for the study trees using 41 visible observations such as tree size, crown condition and the presence of biotic agents. These indexes will be used to identify patterns in the metabolome data associated with Acute Oak Decline (AOD) using machine learning approaches. These patterns will then be integrated with concordant microbiome observations to identify common characteristics of the microbial communities associated with trees across a spectrum of AOD severity.

Pathogen suppressive micro-organisms found in Oak trees.

As well as collecting samples for DNA analysis, during our field campaign, the Bangor University team also collected micro-organisms on Oak for direct study using both traditional and innovative culturing methods. In total, we managed to obtain over 20,000 microbial isolates from 150 healthy Oak trees across the UK. We are currently testing hundreds of these isolates to identify those that can suppress the growth of harmful





Micro-organisms obtained from the Oak trees

Image © Alejandra Orzoñez

micro-organisms for plants. Our main focus is the bacteria *Brenneria goodwinii*, which is the key causal agent of the emerging disease Acute Oak Decline. To do this, we are growing this pathogen together with the Oak microbial isolates on plates containing agar and nutrients needed for their growth. After a couple of days, we inspect these plates looking for any areas where the bacteria *B. goodwinii* has not grown. In this way we have identified three hundred isolates that have pathogen suppressive properties. Additional research is being conducted to taxonomically classify these isolates, and to test their effects in live Oak saplings.

Forest managers' understandings of Oak health and the microbiome

Our social sciences team at Bangor University have been conducting in-depth analyses of UK forest managers' understandings of Oak health and management of Oak woodlands, including an assessment of their perceptions of, and ideas about, the Oak microbiome.

Forest management practices are profoundly influenced by how managers understand problems, the efficacy of solutions, and how these fit with their pre-existing objectives; and alongside a broader investigation of Oak woodland management practice part of this social research has been to consider specific potential microbiome-based interventions to pest and disease threats, such as Acute Oak Decline. This research was built around two principal data gathering exercises, a large-scale survey, and a series of detailed interviews.

The survey was conducted in conjunction with Sylva Foundation's 2021 British Woodland Survey and had responses from over 400 individuals connected to the UK's woodlands, of whom around three-quarters were woodland owners. The work showed that managers are more concerned about the future than they are about the present. Another interesting finding was the diversity of sources managers gather information from; with around one quarter respectively looking to membership organisations, woodland advisors or professional consultants, and their peer network. The first part of this survey work has been written up for publication and is currently under peer review, with a second journal paper planned soon.

Image © Dan Struthers

Coed Aber

Rich qualitative data was gathered from in-depth interviews with a total of 60 forest managers across Britain, the majority of whom maintained woodlands of less than 10 hectares. Many of these interviews were conducted in-situ, alongside the managers in their woodlands. In many cases, our interviewees had an extraordinarily detailed level of knowledge of their woodlands, often drawn-out during walking tours of the woods and demonstrated when discussing the management activities undertaken in their forests at extremely fine scales; often stand by stand or tree by tree. This work will also be published soon.

The project is now entering the final 12 months, and we are working hard to analyse our data and complete the final experiments of the project.

Future Oak team members:

James McDonald (Birmingham University, Bangor University)
Jim Downie (Bangor University)
Alejandra Orzoñez (Bangor University)
Sandra Denman (Forest Research)
Anaparassy Kajamuhan (Forest Research)
Eleanor Curran (Forest Research)
Nathan Brown (Forest Research)
Jasen Finch (Aberystwyth University)
Manfred Beckmann (Aberystwyth University)
John Draper (Aberystwyth University)
Norman Dandy (Bangor University)
Seumas Bates (Bangor University)
Gabriel Hemery (Sylva Foundation)

future-oak.com

Investigating *Gymnopus fusipes* a causal agent of root rot in Chronic Oak Decline

by Bethany J Pettifor, Final Year PhD Student, Bangor University

Gymnopus fusipes, also known by its previous name *Collybia fusipes*, is a common yet extremely understudied root rot pathogen associated with woodland trees across Europe. After being first described in 1783, there is little information on the species until a flurry of research into this pathogen was conducted in France in the mid-1980s, resulting in this mushroom forming fungus being identified as a root rot pathogen of Oak trees. Since this time, *G. fusipes* has been associated with numerous tree host species (including both broadleaf and coniferous varieties) potentially across much of the northern hemisphere. The main host association of this species continues to be with Oak, and *G. fusipes* has been linked with several episodes of Chronic Oak Decline in the UK and similar episodes of decline in Europe.

Beneath the ground level on infected roots, *G. fusipes* infection presents as orange lesions surrounding dark necrotic tissue, and there are often white mycelial fans throughout. However, above the ground level, *G. fusipes* infection frequently presents no symptoms. The characteristic crown decline and fruiting body presence at the base of the trunk, that would be expected with an infection such as this, is often missing, even with a severe infection, and infected hosts repeatedly go undiagnosed until mortality. When fruiting bodies are present, specialist knowledge is required to be able to provide an accurate identification.

Figure 1: Gymnopus fusipes fruiting bodies on the root of an Oak tree

Figure 2: Gymnopus fusipes fruiting body

Figure 3: Above: Lesions on two saplings 6 months after being artificially infected with G. fusipes
Right: Lesion on the root of an Oak tree infected with G. fusipes

One of the aims of this project was to develop a molecular diagnostic that could be used on environmental samples (fruiting bodies and infected root tissue) without the need for culturing the pathogen in the lab, which in this case could take weeks. Throughout this project, I worked to develop a diagnostic tool, specific only to *G. fusipes*, to help identify infection in the field. A process using quantitative PCR (qPCR), similar to the Covid-19 PCR test, was developed to accurately detect *G. fusipes* in fruiting bodies, and infected woody tissue. This test could now be used to identify active infections in trees, even those with no above ground symptoms, as well as being used to confirm the identity of suspected *G. fusipes* fruiting bodies when present. Although the analysis must be carried out in a lab setting, the samples can be collected by non-specialists, including landowners and land managers. This tool represents an important breakthrough in the diagnosis and monitoring of this root rot pathogen, potentially on a global scale.

Another aim of this project was to investigate exactly how *G. fusipes* causes infection through the use of gene expression analysis. For this, we firstly visited an infected site in the UK where mature trees are infected with *G. fusipes* and took root tissue samples.

Then we ran a sapling infection trial, where a number of Oak saplings were subjected to *G. fusipes* infection. After around 6 months, the outer bark was removed, and lesions caused by the pathogen were observed. Lesions forming on the saplings were very similar to the lesions observed on the mature trees out in the field, and tissue from the edge of the lesions was taken for further analysis. For the gene expression analysis, otherwise known as transcriptomics, RNA was extracted from the samples and was sent to be sequenced. Once this data has been analysed, it can be used to determine which genes are being expressed in a new infection (the saplings) and an established infection (the mature trees) and can also highlight differences between the two. This will help establish the mechanisms of infection used by this pathogen to firstly establish an infection in the host, but also then what occurs at a molecular level when *G. fusipes* is actively breaking down host tissues. This will provide a deeper understanding of the infection biology of this pathogen, aiding in the development of management techniques.

Working to protect the UK's Oaks

by Rebecca Gosling, Conservation Evidence Officer Tree Health, Woodland Trust

The Woodland Trust is the UK's largest tree and woodland conservation charity. As an evidence-led organisation, we use the latest research to inform our policy and land practises. To progress our understanding and management of Oak we fund and collaborate on research projects, advise as stakeholders and engage in citizen science.

Learning from other landowners about their experiences with oak declines, such as these Oaks at Exbury gardens

Oak research

The Woodland Trust has the pleasure of funding excellent research projects to aid our understanding of woods and trees. We fund projects with applied outcomes to help us better protect these important habitats. Our current funded projects include research at the Birmingham Institute of Forest Research's (BIFoR) Free Air Carbon Dioxide experiment, where researchers are using underground images to monitor the growth and death of roots of mature Oak trees. In this experiment trees are experiencing increased levels of atmospheric carbon dioxide, this will provide us with insight into how Oak will react to future atmospheric projections. We also support two exciting Oak projects at Aberystwyth University. One of these projects is exploring the changes in soil fungal populations in the rooting space of veteran Oaks. This research will help test the current 15m root protection zones and their effectiveness in protecting veteran Oaks and fungi. The second project at Aberystwyth University is an Oak PhD project. An article on this project was published in the 2021 Action Oak newsletter, demonstrating how their research is utilising modelling tools to investigate the vulnerability of sessile Oak woodlands to the effects of climate change. In the past we have also supported the PuRpoSE Oak ecosystems project and Acute Oak Decline research.

We also advise on many research projects as stakeholders. This includes the NERC-funded Treescapes project: newLEAF. This project is investigating the adaptability of trees, including Oak. As stakeholders we provide input on how the outputs can be used in conservation policy and practise.

Citizen science

To generate valuable data and increase awareness we also engage in important citizen science projects. We are a part of the multi-partner **Observatree project** [1] which uses a citizen science approach to tree pest and disease early warning. We

Oak processionary moth surveying

Observatree training day at the Yorkshire Arboretum delivered by Fera Science Ltd

have 200 volunteers, who are managed by the Woodland Trust and trained by Forest Research and other partners, that monitor their local trees for key pests and diseases. These include Acute Oak Decline, Oak lace bug, and Oak processionary moth. The volunteers are a big support in our efforts to reduce the spread of Oak processionary moth. Each year they survey sites across the Oak processionary moth buffer zone and feedback any findings to both the Woodland Trust and Forest Research. This helps us in our management of this Oak pest as site managers then arrange for appropriate control action. The volunteers have also aided Acute Oak Decline research, recording some of the most northerly and westerly findings and sending in swabs and samples to the Forest Research Acute Oak Decline team. In addition, we also run the Ancient Tree Inventory, a citizen science project mapping the UK's ancient and veteran trees. This map includes records of 84,106 Oaks, of which 6,421 trees are classified as ancient! Knowing where ancient Oak trees are is the first step in protecting them!

Publications

This year, alongside the National Trust and Green Recovery Challenge Fund, we published an **assessment guide for ancient and veteran trees**[2] (Gilmartin, 2022). This important guide will aid landowners to protect their trees, such as the ancient Oaks listed on the Ancient Tree Inventory. We also published our long-awaited **Woodland Creation Guide**[3] (Herbert *et al.,* 2022) with an accompanying **Tree Species Handbook**[4] (Hotchkiss and Herbert, 2022). This handbook contains information on pedunculate and sessile Oak, providing practitioners key information for planting the right tree in the right place.

Oaks are such an important part of our landscape, we aim to continue growing our work, working with the Action Oak partnership to ensure their protection.

1 **observatree.org.uk**
2 **woodlandtrust.org.uk/media/51153/ancient-and-veteran-trees-an-assessment-guide.pdf**
3 **woodlandtrust.org.uk/media/50673/woodland-trust-woodland-creation-guide.pdf**
4 **woodlandtrust.org.uk/media/50812/tree-species-handbook-woodland-creation-guide.pdf**

Action Oak report

by Dr Estrella Luna-Diez, Birmingham University, BIFoR

The research group led by Dr Estrella Luna-Diez is based at the University of Birmingham and the Birmingham Institute of Forest Research (BIFoR). The team works within the framework of BIFoR of finding solutions to mitigate the impacts of invasive pests and diseases in forest trees. The group works mostly with Oak trees and currently three main lines of research are being pursued:

Understanding the impact of elevated carbon dioxide (eCO_2) against powdery mildew (PM).

Led by PhD student Mark Raw and funded by a Leverhulme PhD Forest Edge studentship

Levels of atmospheric CO_2 are at the highest point in 2 million years due to human activity. Whereas we know that eCO_2 can increase plant growth, its effect in plant immunity remains highly controversial. We are investigating how eCO_2 impacts resistance to PM in both mature Oak trees and seedlings. This research is based at the Free Air CO_2 Enrichment (FACE) facilities, providing a unique tool to understand the impacts of eCO_2 in trees under their natural environments. We have shown that eCO_2 causes seedlings to become more susceptible to the infection, which could have devasting future impacts (Sanchez-Lucas *et al.* – BioRxiv). Mature trees seem to tolerate annual infection by powdery mildew. To understand how eCO_2 impacts mature trees we have taken monthly leaf samples from the canopy throughout the year to evaluate the incidence of PM. Using metabolomics, we are investigating the effects of eCO_2 on leaves from mature trees collected from May-September and in seedlings with contrasting levels of PM infection (i.e. healthy or infected). We have identified unique metabolite profiles that mark resistance and susceptibility to the disease in mature trees and seedlings.

Reference: Elevated CO_2 alters photosynthesis, growth and susceptibility to powdery mildew of Oak seedlings. Rosa Sanchez-Lucas, Carolina Mayoral, Mark Raw, Maria-Anna Mousoraki, Estrella Luna. doi: ***https://doi.org/10.1101/2023.01.07.523094***

Investigating chemical-induced priming of defence in Oak seedlings against powdery mildew (PM).

Led by postdoctoral fellow Rosa Sanchez-Lucas and funded by the JABBS Foundation

Plants are continually exposed to multiple stresses that are currently more frequent in a climate change scenario. However, plants have developed highly sophisticated strategies to face these threats. One strategy is the priming of defence, a sensitisation of plant defence mechanisms that allows for a faster and/or stronger defence mechanisms activation after subsequent attack. Many chemicals can trigger priming wide-spectrum effectiveness. However, studies in Oak seedlings are lacking. This work aims to determine whether Oak seedlings can express chemical-induced priming and the potential trade-off in the growth. We are using well-characterised priming elicitors such as salicylic acid (SA), jasmonic acid (JA) and β-aminobutyric acid (BABA). Moreover, through metabolomics and transcriptomics, we are investigating the mechanisms of priming of those compounds. Our results will provide valuable information to fight PM

University of Birmingham research group

disease in Oak seedlings, as it is considered a bottleneck for woodland regeneration.

Understanding epigenetic mechanisms that contribute to an immunological memory against biotic stress.

Led by postdoctoral fellow Rosa Sanchez-Lucas and funded by the NERC Treescapes grant MEMBRA (membra.info)

Plant pests and diseases, both endemic and recently emerging, are spreading and exacerbated by climate change. For example, in recent years, Acute Oak Decline (AOD) has emerged as a disease endangering Oak landscapes. Plant epigenetics has recently acquired extraordinary interest as it has been shown to contribute to both short-term phenotypic plasticity and the longer-term adaptive capacity of plant responses to, including the capacity to transmit these marks to progenies. Our work aims to study how pest and disease pressures alters DNA-methylation imprinting in Oak. For this, trees were scored and classified in severity levels of AOD and defoliation caused by the Oak winter moth (OWM) caterpillars. Leaf-DNA was extracted and subjected to Whole Genome Bisulfite Sequencing (WGBS). Bismark software and R scripts (DSS., DMRcaller) were employed to analyse methylomes. Differentially Methylated Regions (DMR's) were observed in different C-context. Correlation analysis is currently being used to identify global and targeted changes in DNA methylation with AOD disease insect defoliation severity. The identification of epigenetic mechanisms marking Oak resilience could be used to reforestation and conservation of future forests in a hostile environment.

Oak powdery mildew and tree health in the Celtic rainforest

by Octavia Hopwood, Aberystwyth University

One of the primary aims of this project was to investigate tree health within the Temperate Rainforest habitat in Wales (also known as the Celtic Rainforest), with specific focus on the fungus that causes Oak powdery mildew, *Erysiphe alphitoides*. Although generally not a life-threatening disease in mature Oaks, powdery mildew can severely impact young tree growth, particularly in saplings and young specimens. This is a concern for future regeneration of Oak trees, particularly given their diminishing numbers and other threats that may impact Oak health and abundance. Understanding more about the disease and tree health in general, will help direct future research into the subject and potentially impact efforts to safeguard young trees and the future of Oak.

In preliminary trials with young Oak saplings, which were subjected to a range of treatments simulating climatic conditions, a trend emerged where saplings treated with nitrogen fertiliser showed higher severity of powdery mildew. This result was of interest because, with an increase in agricultural emissions, rates of nitrogen deposition over the country have also increased. A further experiment, looking into nitrogen deposition on a larger scale was initiated. Ammonia and NO_2 samplers were deployed at woodland sites across Wales, half of which were within areas classified as temperate rainforest, and half were placed in Oak woodlands not classified as temperate rainforest habitat.

Nitrogen samplers

The locations of the two groups showed a clear east/west divide, with rainforest sites located in the west of Wales, and non-rainforest sites located in the east of the country.

The samplers showed that there was a clear divide between the two groups of woodlands in terms of atmospheric nitrogen. Temperate rainforest sites showed much lower concentrations of both ammonia and NO_2, whereas non-temperate rainforest sites showed higher concentrations. Given the east/west divide between the two woodland groups, we concluded that the higher levels of nitrogen seen in the non-rainforest woodlands, were due to higher agricultural

Octavia putting out air sampler

Powdery mildew

activity in the area. In contrast, the west of Wales (where the rainforest sites were located), is generally more mountainous, and thus large scale, intensive agriculture may be harder, reducing atmospheric nitrogen pollution. Furthermore, with a south-westerly wind from the Atlantic, cleaner air may be blowing over the westerly sites and reducing the nitrogen deposition.

One specific factor of health that we were interested in was mycorrhizal fungi since they form symbiotic relationships with the trees and aid nutrient and water uptake. Using DNA metabarcoding, we analysed leaf and soil samples from our woodland sites to investigate the diversity of fungi present. Two patterns emerged from the data. The first pattern saw higher amounts of Oak powdery mildew, *Erysiphe alphitoides*, present in non-rainforest sites. Generally, the spread of *Erysiphe alphitoides* has been in a north-westerly direction across Britain, which may explain this result. We also observed that the rainforest sites showed nearly double the abundance of mycorrhizal fungi than in the non-rainforest sites. These two observations were not necessarily associated to each other, however they did both show correlation with the amount of nitrogen deposition.

Given that nitrogen is often a limiting factor in woodland ecosystems, there is the potential that in areas with higher amounts of nitrogen deposition, the ecosystem is shifting accordingly to the conditions. With higher levels of nitrogen, plants often produce softer, leafy growth, which is appealing to the powdery mildew. In addition, if trees are gaining their required nitrogen from external sources, the symbiotic relationships between trees and fungi may not need be so strong, and thus fungi numbers may start to decline. Further interactions between mycorrhizal fungi and fungal diseases may be altering tree immunity, however these interactions are more difficult to interpret.

This experiment was conducted as a preliminary test to determine whether further exploration should be conducted and highlights the benefits of DNA metabarcoding in exploring whole ecosystem dynamics. Following the results that were obtained, a more extensive experiment will be conducted in the growing season of 2023 to provide more reliable results. It is hoped that the new results will confirm our previous conclusions and enable further channels of research.

Elucidating heart-rot fungal communities

by Rich Wright and Lynne Boddy, Cardiff University

Heart-rot is the decay of central tree tissues (heart wood) that are no longer functional in conduction of water from roots to leaves. It should not be seen as a disease since the tissues are already dead, although a few heart rot fungi, such as *Heterobasidion* species in conifers, do have the ability to kill living tissues and hence invade functional sapwood. Heart wood is important habitat for specialist heart-rot fungi, including rare taxa such as *Buglossoporus quercinus* in Oak. Heart-rot is hugely important as habitat for invertebrates, over 1700 species depend on this type of decaying wood in the UK alone; and over 1000 species of birds and mammals worldwide use hollow trees as habitat (Boddy 2021).

Despite the desire to understand heart-rot since the middle of the 19th century, little is known about the fungal communities involved. Fruit bodies on the outside provide some clues, but they can be misleading. Studies on beech (*Fagus sylvatica*) revealed that the actual fungal communities in the centre of the trees comprised very different wood decay species than fruit body surveys suggested (Boddy, 2021; Gilmartin, 2020).

Photo © Rich Wright

Beefsteak fungus (Fistulina hepatica) fruiting on Oak

Nonetheless, information on fungal fruit bodies on Oak is a valuable starting place. Information can be gleaned from the literature and records databases. However, these tell little about the relationship between fruit bodies and tree size and condition. To get a better handle on this a community science project has been launched (Wright *et al*., 2022). Anyone with a smartphone or PC can participate and we would value all the records that we can get. Details are on the project website at **foreverfungi.co.uk/oakfungisurvey**.

Fruit body surveys on Oak in southern Britain seem to be dominated by the beef steak fungus (*Fistulina hepatica*), followed by chicken of the woods (*Laetiporus sulphureus*), with fewer records of *Ganoderma australe* and *Grifola frondosa*. But are they actually the main decayers of the heartwood? To answer this question we have so far extracted cores, from 42 *Quercus robur*, 37 *Quercus petraea,* and 7 hybrid trees greater than 170cm circumference from 8 sites across Wales and Southern England. From each tree, a small piece of bark was levered off the trunk at 1.5m above ground, a 6mm wide core was extracted using a cleaned and flame-sterilized 45cm long increment borer. The bark was then replaced. Each core was transferred to the lab in a plastic drinking straw, where chips were removed at 1cm intervals and plated onto agar. The fungi that grew out were grouped together based on mycelial morphology, and each was identified using Sanger amplicon sequencing.

Unlike Beech, the wood decay basidiomycetes bringing about heart-rot

Photo © Rich Wright

Top left and right: Extracting wood cores from veteran Oak
Right: Extracted Oak wood core

Photo © Rich Wright

Cultures of fungi from Oak cores

in Oak reflect fruit body surveys. So far, about 40% of trees had *F. hepatica*, 26% *L. sulphureus* and 10% other basidiomycetes rotting the heartwood. *F. hepatica* occupied the greatest volume. *L. sulphureus* was mostly found in central regions. As well as basidiomycetes, over 50 ascomycetes have been detected, some of which are commonly present. Ongoing work involves identification of fungi in heartwood by direct extraction of DNA followed by Sanger sequencing and Next Generation Illumina sequencing. The possible directions of change in heart-rot communities are being evaluated by pairing different species against each other in artificial culture under different conditions, and in wood. Also, the ecophysiology of dominant basidiomycetes is being studied by determining effects of temperature, water potential, pH and tannins on growth in artificial culture.

References

Boddy L (2021) *Fungi and Trees: Their Complex Relationships*. Arboricultural Association, UK.
Gilmartin EC (2020) *Fungal Communities of Beech Trees: Heart Rot and Origins of Decay*. PhD thesis, Cardiff University.
Wright RA, Finch J, Brown N (2022) Oaks and fungi in the UK. *The Best of ARB Magazine* 50 – 57.

Support

This project is funded by a KESS 2 studentship, and supported by the Welsh Government.

Arming against *Armillaria* – leveraging antagonistic fungal interactions as biocontrol of *Armillaria*

by Ed Pyne, Bangor University

Armillaria spp. (left) and Hypholoma (right) battle for control over an Oak log (Ashridge Estate - Oct 2022)

Honey fungus (*Armillaria* spp.) is perhaps the most notorious fungal pathogen of Oaks. Several species of *Armillaria* exist in the UK, some are mostly benign wood decomposers directly benefiting their surrounding ecosystem while others are highly damaging and destructive pathogens. *Armillaria* infections are notoriously difficult to control, most current control methods are ineffective, expensive, and difficult to implement.

Wood inhabiting fungi are fiercely territorial within wood leading to highly combative interactions, there is scope to exploit these antagonisms to combat pathogenic *Armillaria* species in the UK. My research focuses on the exploitation of these antagonistic interactions to develop a cost effective, ecologically sympathetic biocontrol for pathogenic *Armillaria* species impacting Oak within the UK. The sulphur tuft fungus *(Hypholoma fasciculare)* has previously been demonstrated to show efficacy in *Armillaria* infected Douglas fir stands in North America, but the lack of knowledge surrounding this interaction, specifically in a UK context, severely inhibits the deployment of the *Hypholoma* as an *Armillaria* biocontrol.

The first step of our research confirmed the effectiveness of *Hypholoma* at displace *Armillaria* in small Oak sapwood blocks under controlled conditions, sometimes leading to displacement in as little as 14 days! In a next step, we assessed the bouquet of volatile chemicals produced during these fungal fights. Volatile chemicals are known to influence these interactions, analogous to the use of chemical weapons in human wars. Characterisation of these compounds not only aids in unravelling the mechanisms driving the outcome of this interaction but may aid in the development of chemical-based control methods that mimic nature.

We are also using RNA sequencing to characterise the interaction between *Armillaria* and Hypholoma at the gene expression level. By analysing the genes used during this interaction we will gain a deep understanding of how Hypholoma is able to displace Armillaria so efficiently. We will identify the key genes and biological processes underpinning the outcome of the antagonism.

Our characterisation of this interactions utilises several independent methodological approaches, traditional culture techniques, volatile compound analysis, molecular characterisation and microscopy that collectively will paint a full picture of the interaction mechanisms. This data will provide the backdrop from which to develop well designed field experiments and provides the foundational work required for the implementation of this potentially very effective biocontrol agent of *Armillaria* in the UK.

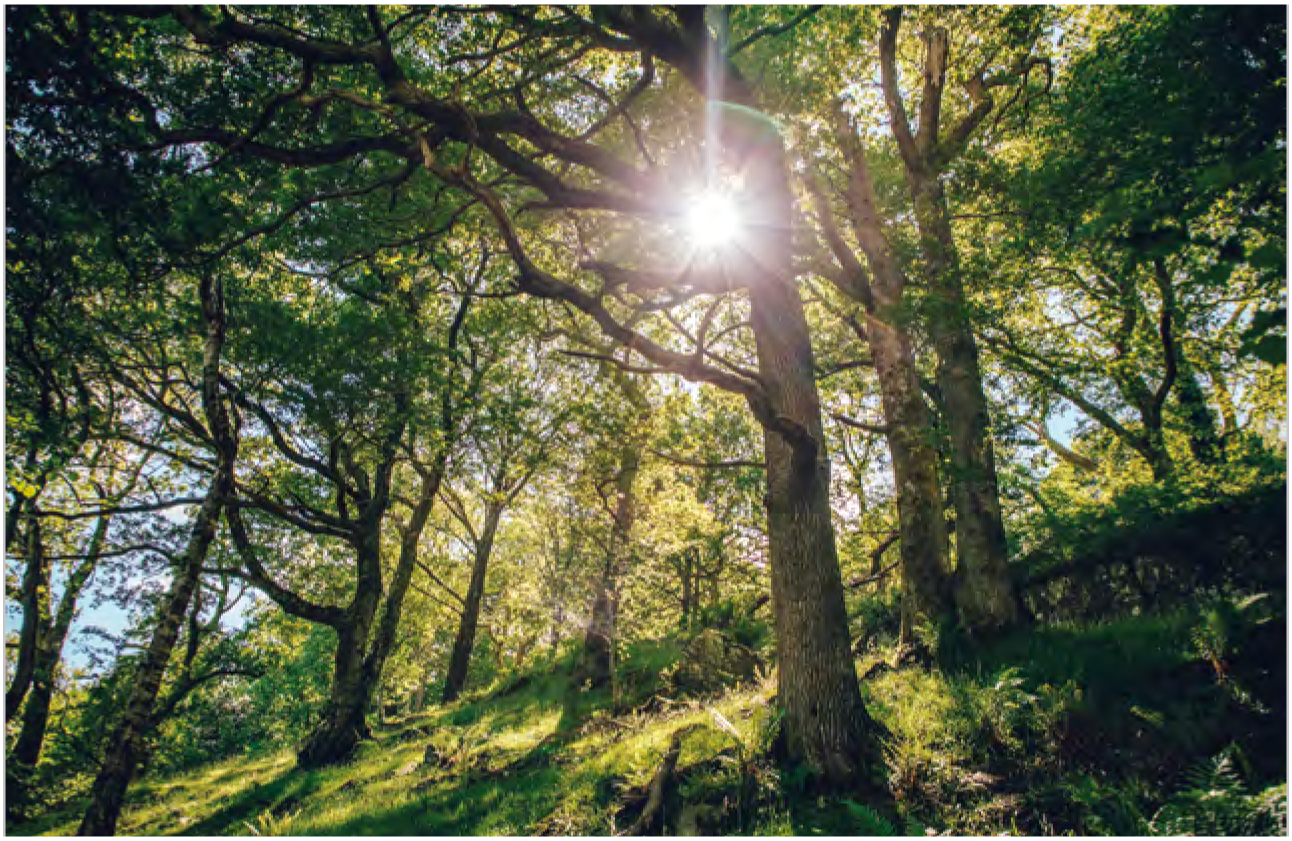


Image © Dan Struthers

Coed Aber

Reducing the impact of climate change-induced storm events on native UK Oak health

by Kate Halstead, Newcastle University

Oak trees are an essential component of the UK's treescape, yet, in recent years, their population has declined due to several threats. Extreme storm events have been identified as a significant abiotic threat to the health of native Oaks, causing severe damage and mortality. Due to climate change, storm events are increasing in intensity and occurring in regions that were once unaffected, where Oak trees have less resilience to extreme windspeeds.
The UK Winter 2021/22 storm season was particularly damaging regarding event occurrence and intensity. Over the season, the UK Met Office Storm Centre recorded seven named storms. Storm Arwen caused extensive damage to Oaks in the North of England and Scotland. Extreme windspeeds came from an unusual North Easterly direction. As the predominant UK wind

Above: Windthrown Sessile Oaks at the Wallington Estate

DJI Matrice 300 RTK drone with GeoSLAM LiDAR scanner

direction is from the South West, the root plates of many Oaks had little resilience, which caused uprooting.

This research aims to profile the consequences of Winter 2021/2022 extreme storm events upon the health of the UK's native Oak population, studying Oak in different settings such as woodland, parkland and field margins. The unique wind direction of Storm Arwen presented an opportunity to study the interrelationships between Oak health and strong wind in the North East of England. My three local sites are (1) The Wallington Estate, Northumberland, (2) Cockle Park Farm, Northumberland and (3) Gosforth Nature Reserve, Tyne and Wear. Fieldwork commenced earlier this year. Across all three sites, I have recorded a total of 82 storm-damaged Oaks. Damage was classified into three main categories: crown damage, windthrow and windsnap.

The research will explore the field of dendrochronology, more commonly known as tree ring analysis. An increment borer was used to take a small 20cm core from each damaged tree. To date, I have collected over 160 cores. Analysis of the cores can be used as an indicator of past productivity and tree health. This will help to estimate the baseline health of the damaged Oak, prior to the storm event, and determine if existing health conditions contributed to storm damage. Until now, the existing health of Oaks prior to storm damage is a factor that has been overlooked.

Novel remote sensing techniques will also be incorporated into the project. Remote sensing is the acquisition of characteristics of an object by measuring its emitted and reflected radiation from a distance e.g., satellite or drone. A 3D laser scanner (GeoSLAM ZEB Horizon), and a quadcopter drone mounted with a LiDAR sensor, have been used to create high-resolution 3D models of the structure and canopy of the damaged Oak.

Analysis of the structure will help to determine the extent to which local wind strength and direction are responsible for storm damage. Whilst scanning, I have also been recording the health metrics of the damaged Oaks including crown condition, DBH, signs of decay, disease, bark stripping, branching structure and exposed root plate dimensions.

Extreme wind can be considered a disturbance agent, breaking up canopies and creating regeneration niches and deadwood. Following recent storms, landowners such as the National Trust have opted to leave damaged Oak and deadwood in situ, where possible. This aims to increase forest diversity and provide wildlife-rich deadwood. At each site, a GeoSLAM ZEB Horizon LiDAR

3D point cloud of storm damaged Oak using a ZEB Horizon scanner

scanner has been used to derive the amount of carbon lost from the canopy and stored in deadwood. This is significant, as Oak trees have the potential to store more carbon than any other native UK tree species.

The next stage of the research will involve the analysis of the tree ring cores and remotely sensed point clouds. Analysis of both will identify site-specific 'predictor variables', those factors which are most likely to predispose Oak to damage during storms. An understanding of which factors contribute to damage during extreme storms will provide Action Oak with suggestions to alleviate stresses and aid Oak management practices. Successive storms can cause sequential damage, through increasing exposure to damaging winds and fatiguing effects on root and stem strength. I will therefore document any future damage at my sites caused by potential Winter storms in 2022/23 and 2023/2024.

This is a 3.5-year PhD research project, based at the School of Natural and Environmental Sciences, Newcastle University, under the supervision of Dr Rachel Gaulton, Dr Roy Sanderson, Dr Andrew Suggitt and Prof. Christopher Quine. This research is funded by the Action Oak initiative.

For further information please contact
Kate Halstead
K.E.Halstead2@newcastle.ac.uk
at Newcastle University.

References

Brown, N., Vanguelova, E., Parnell, S., Broadmeadow, S. and Denman, S., 2018. *Predisposition of forests to biotic disturbance: Predicting the distribution of Acute Oak Decline using environmental factors.* Forest Ecology and Management, 407, pp.145-154

Quine, C.P., Atkinson, N., Denman, S., Desprez-Loustau, M-L., Jackson, R., Kirby, K. (eds) 2019. *Action Oak Knowledge Review: an assessment of the current evidence on Oak health in the UK, identification of evidence gaps and prioritisation of research needs.* Action Oak, Haslemere, UK.

Quine, C., Gardiner, B., Moore, J. 2021. *Wind disturbance in forests: The process of wind created gaps, tree overturning, and stem breakage.* Plant Disturbance Ecology.

The drivers of Oak masting in the UK
Year 2 annual report

by Ryan McClory, University of Reading

Introduction

Years of low seed crops punctuated by occasional bumper crops, termed “masting” or “mast seeding”, is a common reproductive strategy in perennial anemophilous plants (Fernández-Martínez *et al*., 2019; Kelly & Sork, 2002). Masting provides ecosystems a sudden abundance of resources for consumers and influences flora and fauna across trophic levels (Elkinton *et al*., 1996; Ostfeld & Keesing, 2000) including influencing human disease risk (Jones *et al*., 1998; Ostfeld, 1997; Ostfeld *et al*., 2001).

Several non-exclusive hypotheses have been put forward to explain the evolutionary (Kelly, 1994) and mechanistic (Koenig, 2002; Pearse *et al*., 2016; Satake & Iwasa, 2000) underpinnings of masting. However, there remains much discussion and debate. For instance, studies on the meteorological factors of masting still lack consensus, as evidenced by contrary results between species (Bogdziewicz *et al*., 2019; Pérez-Ramos *et al*., 2015) and within species from different studies (Lusk *et al*., 2007; Sork *et al*., 1993).

Unpredictable seed crops combined with poor seed storage behaviour - i.e. recalcitrant seeds (Berjak & Pammenter, 2008; Roberts, 1973), can present a novel problem for tree regeneration and planting strategies. Due to a failed acorn crop across the UK and much of Europe in 2021, this led to a shortage of pedunculate Oak (*Quercus robur L.*) and sessile Oak (*Quercus petraea* (Matt.) Liebl.) from nurseries for the 2022/23 planting season (Bole, 2022). This demand can be met by importing acorns from elsewhere in Europe, but some foreign acorns are not well adapted to the British maritime climate and tend to result in lower quality trees (i.e. reduced height and survival) (Hubert, 2005). As such there is an alignment between the needs of the UK forestry industry with the academic interest of discovering the mechanistic drivers of masting.

The PhD study

My doctoral research focusses on the putative drivers of Oak masting in the UK, with the aim that this better understanding may contribute to a more consistent acorn crop for conservation and forestry. My research objectives focus on explaining variation in acorn production of individual trees within and between years, analysing how nationwide weather cues may drive masting, and exploring what methods can be undertaken to increase acorn production. I list these areas of study, as well as the progress made, below.

AREA 1: How is individual variarion in acorn crop a product of the abiotic and biotic environment?

Masting normally infers large geographic synchrony in acorn production across populations. This view is simplistic, as even during masting events the majority of the crop may be made up of contributions only from the most fecund trees (Greenberg, 2000; Koenig *et al*., 1994; Pérez-Ramos *et al*., 2014). By assessing the drivers of this individual variation, we may gain insight into the mechanistic underpinnings of acorn production.

Agricultural practices are moving towards precision farming techniques, which do not assume homogenous conditions across the whole field (Kent Shannon *et al*., 2018). If the same can be expected for woodlands, then study of variation in soil nutrients, microclimate, genetics, and canopy growth/structure amongst individual trees could prove particularly informative to explain the drivers of masting. The influence of these different factors is likely to vary temporally in their impacts and interactions. As such, these factors will be considered simultaneously and throughout the whole reproductive cycle of the individual acorn, from bud initiation to seed fall. I have been undertaking a multi-year and multi-factorial observational study between individual trees of the same population at Wytham Woods, Oxfordshire. Key hypotheses include:

1) Trees with flowering phenology that is more synchronised with the population mean will have an increased acorn crop;
2) Individual trees with a smaller acorn crop this year will have had a higher proportion of post germination flower abscission or early acorn abortion;
3) A trade-off between vegetative and reproductive growth within the canopy will be observed;
4) Highly productive trees will differ genetically from less productive trees.

Progress and next steps

I have a well-established field site at Wytham Woods, Oxfordshire, where I have been recording acorn production of 40 trees spread across the site.

- Acorn numbers have been assessed visually for 2020, 2021 and 2022. I have been regularly collecting from 160 litter traps (Figure 1), set up in 2021, which provide numbers of mature acorns, aborted acorns, Oak flowers, and leaf litter. Counts and collections will continue in 2023.

Figure 1: An experimental tree at Wytham Woods, surrounded by four litter traps placed in the centre of the crown at the four cardinal directions

Figure 2:
Canopy photos taken throughout the season from experimental tree 21.
The dates the photos were taken are as follows: (1) 30/03/21, (2) 20/04/21, (3) 20/05/21, (4) 02/06/21, (5) 16/07/21 and (6) 31/08/21

- In Spring 2022 my 40 experimental trees were sampled for genetic sequencing as part of the Darwin Tree of Life Project. This will provide information on how genetic variation can affect acorn production and tree phenology.
- Temperature and humidity loggers (Tinytag, Gemini Data Loggers, Chichester, UK) attached to each of the 40 trees in Autumn 2020 have collected data continuously. In August 2022, they were collected to have the battery replaced and general maintenance performed before being placed back out in the field.
- Canopy photos have been consistently taken for my forty trees throughout the project (Fig. 2) to provide a record of leaf phenology. Photos are sorted and converted (from .RAW to .JPG) before analysis. The canopy analysis software (WINscanopy, Regent Instruments, Quebec, Canada) has provided metrics of crown growth and structure such as leaf area index and crown porosity from these images. This process is ongoing as I continue to collect canopy photo data.
- I am going to collect soil samples in late Winter of 2022/23 to determine soil textural classification, pH, cation exchange capacity and exchangeable cations.
- I will use the Planet database of satellite imagery (3m Å~ 3m resolution) to assess individual tree and population phenology. Additionally, I shall use multispectral imagery from drones flown over Wytham Woods in 2022/23.

AREA 2: Can we model the influence of the weather on acorn production in *Quercus Robur* and *Quercus Petraea*?

Weather is considered a key driving factor in mast seeding, due to its ability to act as a large-scale synchroniser (Bogdziewicz *et al*., 2017, 2021; Norton & Kelly, 1988). Recent studies of weather's influence on masting give varied, and occasionally contradictory, results with differing species specific responses to the same weather events (Bisi *et al*., 2016; Bogdziewicz *et al*., 2019; García-Mozo *et al*., 2007; Koenig *et al*., 2016). The differing reactions to weather cues for tree seed production reported in the literature may in part come from:

- between-species variation in their life history and reproductive cycles (Bogdziewicz *et al*., 2019; Pérez-Ramos *et al*., 2015);
- and/or from reactions to weather cues changing depending on the climate (Masaki *et al*., 2008; Mooney *et al*., 2011; Poncet *et al*., 2009);
- and/or the reproductive stage of the tree when the weather cue occurs (Allen *et al*., 2017; Buechling *et al*., 2016).

Species-specific studies considering the effect of the environment at each phase of development of a masting species reproduction are now required. I have been analysing records of acorn production at several sites to determine the role of weather in masting for Oaks.

Key hypotheses to test include:
1) A combination of climatic conditions and remote sensing data can be used to predict mast years for Oak dominant woodlands in the UK;
2) The frequency of mast years in the UK will be affected by climate change;
3) Masting in UK native Oaks will react differently to weather cues than those found in continental Europe.

Progress and next steps
The past year involved the consolidation of my data sources and the start of my data analysis.

- I have amassed a database of ten sources of long-term time series for *Quercus robur* and *Quercus petraea* in the UK, with the two longest times series over 20 years in length, and one of 44 years (and still going). I have 100+ times series across Europe, with four above 40 years in length and many more being above 20 years.
- I have retrieved environmental data for each of these sites from the European climatic data base. Additional environmental data was retrieved for the UK sites from nearby weather stations. This has provided me with ten unique weather indices, organised into monthly values for the 33 months prior to acorn production date.
- I developed a statistical methodology to analyse the influence of these weather variables on acorn production. As the weather variables were not independent, prone to collinearity, and often more numerous than the number of observations, I used a mix of statistical methods to identify and interpret the role of weather variables in determining acorn production: (1) partial least squares regression (PLSR), (2) random forest (VSURF) and (3) general linear models (GLM).
- I tested the model on the longest UK time series I had available and wrote it up as a methods and results section. I now plan to expand this methodology to, first, all my other UK time series and then many of my European data sources.

AREA 3: Does pollen source matter: Testing pollen addition methods to improve acorn production in *Quercus Robur*

An overview of pollen flow studies presents a consensus that around 50%-70% of the geneflow comes from pollen outside the stands studied (Dow and Ashley 1998a, b, Streiff *et al*. 1999; Craft and Ashley 2010; Abraham *et al*. 2011). This majority of foreign male parentage remains consistent amongst studies on clonal seed orchards (Buiteveld' *et al*. 2001), naturally-regenerated stands (Dow and Ashley 1998a; Streiff *et al*. 1999), in stands with nearby pollen sources (Dow and Ashley 1998b), and those without nearby pollen sources (Craft and Ashley 2010). Moreover, whilst each of these studies analysed stands of similar size (four to five hectares) the individuals in each study varied from 57 to 296 adult trees, suggesting the pattern is not driven by the number of potential pollen donors.

Looking at within-stand pollen flow success, Dow and Ashley (1998b) found proximity to pollen source to not be as influential as traditional models would assume. Often trees would fail to pollinate their nearby neighbours but instead pollinate much more distant trees, whilst genetic analysis of shared alleles of study trees (a proxy for relatedness) had no impact on predicting acorn production.

Genetic analysis of acorns can only determine pollination on the basis of a successful outcome, i.e. acorns that formed from foreign pollen; but not whether there is higher initial foreign pollen load or later selective abortion of non-foreign acorns. To distinguish the reasons for higher out-of-stand pollen success we need studies that manipulate pollen source and quantity experimentally.

I set up an experiment in Spring 2022 to test successful acorn production as a response to supplementary pollen. The research aims to determine the effect of pollen source on *Quercus robur* acorn development and abortion. The following hypotheses shall be tested:

1. There will be a selective abortion of acorns dependent on pollen source, with acorn production favouring out-of-stand pollen;
2. The ratio of out-of-stand and within-stand pollen will affect flower abortion and mature acorn production.

Progress and next steps

- I selected trees for analysis at Wytham Woods, Oxfordshire and then selected 300 branches across these trees to perform the experiment on. This included trees with lower branches reachable from the ground, but also included branches in the upper canopy of trees reachable via the canopy walkway (up to 17m above ground) at Wytham Woods. Each selected branch was isolated from the outside environment via pollen bags (PBS International, Scarborough, UK).
- Shortly after budburst the Oak catkins were collected from trees outside of the experimental population. Collection was made from five sites across the south-west of England, as well as at Wytham Woods, Oxfordshire. These flowers were then dried, and pollen extracted from them.
- Pollen tube growth was measured for the collected pollen to check it was viable. Once the female flowers were receptive, the collected pollen was added to them within the following treatment groups:
  - Within Stand Pollen (WSP);
  - Out of Stand Pollen (OSP);
  - A 50/50 mix of within stand and out of stand pollen (50/50);
  - Natural pollination (NAP);
  - No pollination (NOP)
- These flowers were closely monitored over the growing season to determine if they did, or did not, develop into acorns; and if so, how far acorn development progressed. At the time of writing, acorns remain maturing on the trees. Early results provide more developing acorns in the out of stand pollen than the other pollination treatments.

Additional activities

In addition to my main three studies, I have been undertaking a mixture of work and training as part of my doctoral training, a non-exhaustive list is written below:

- I presented and passed my Confirmation of Registration Report. This involved creation of a 40-page report, detailing my PhD programme studies to date and my future progress. I passed this and am now registered as a PhD candidate (which means I am able to submit my thesis in due course for the PhD examination).
- I attended the Ecology Across Borders conference in Liverpool, 12th of December to 15th of December 2021. The conference is a collaboration between the British Ecological Society and the French Society for Ecology and Evolution. I also attended and presented at the MASTNET meeting in Liverpool, a workshop and meeting of international researchers focused on studying masting.
- I applied and was selected to attend a seven-day training course "Tracking the Socio-ecology of Southwestern European Mountain Forests: from the Field to the Space, from the Present into the Future" in Manteigas, Portugal. The course was a combination of remote sensing, forest modelling, fieldwork techniques and discussions with forest stakeholders. It was funded by an EU COST action initiative.
- I have presented my doctoral research at the Action Oak Partner event at Kew Gardens, the Wytham Woods Michaelmas Term Lectures, and to (my old research group) Ayco Tack's Plant Microbe Lab at Stockholm University.
- I am continuing to take part in a multi-continental study comparing direct seeding versus planting for Oak regeneration. I have created and maintained a common garden experiment of *Quercus robur* and *Quercus petraea* seedlings at the University of Reading's Experimental Grounds.
- As a result of connections made at the Ecology Across Borders Conference, I have been given access to the litter trap output from Birmingham Institute of Forest Research's FACE Free Air Carbon Dioxide Enrichment (FACE) experiment. I shall write the results from this up and include in my thesis.
- I took on an undergraduate student as part of the Undergraduate Research Opportunities Program (UROP) with financial support from the University of Reading, designing a project for them involving using canopy photo analysis and acorn counts to create a research poster. Additionally, I have been helping a Masters student within my research group with canopy photo analysis for their project.
- I have volunteered as a reviewer of manuscripts for the journals Forest Ecology and Management and Central European Forestry Journal.
- I was interviewed as part of Wytham Woods citizen science initiative around planting Oaks.

The year ahead

This second year has focussed on collecting data for study 1 and study 3, whilst building the statistical model for study 2. The next year will involve some data collection and retrieval for studies 1 and 3, and the complete write up of study 2.

Acknowledgments

This PhD project is made possible due to the generous funding from the Future Trees Trust, with support from: Patsy Wood Trust, Scottish Forestry Trust, Aitchinson-Trait Trust, in conjunction with Action Oak and the ALA Green Charitable Trust.

References

Full list of references available on request.

Acknowledgements

Action Oak would like to thank the partners who support the vital research and monitoring of the UK's native Oak trees.

Partners
Steering committee

The Duchy of Cornwall
Woodland Heritage
Woodland Trust
National Trust
Royal Botanic Gardens Kew
Forestry Commission
Forest Research
Defra
Scottish Government
Northern Ireland Government
Welsh Government

Three sub committees consist of:
Fundraising and Communication
Knowledge Exchange and Management Practise
Research and Monitoring

Aberystwyth University
Agri-Foods and Bio-Sciences Institute
Animal and Plant Health Agency
Arboricultural Association
Bangor University
Birmingham University (BIFoR)
Cardiff University
Country Land and Business Association
Future Trees Trust
Horticultural Trades Association
James Hutton Institute
Natural England
Newcastle University
Royal Forestry Society
Scottish Royal Forestry Society
Smallwoods Association
The Tree Council
UK Squirrel Accord
University of Liverpool
University of Reading
University of the West of England

Action Oak

Safeguarding survival

Action Oak works with partners to fund the vital research and monitoring needed to better understand the threats facing the UK's native Oak trees, enabling our partners to devise real solutions that allow us to safeguard the survival of our Oaks for generations to come.

Why is it important?

A prominent tree across history for the UK's flora, fauna & human population, the benefits of this native tree have been felt far and wide, from the biodiversity that they support to the livelihoods of the people who rely on them.

Oaks are fundamental to our landscape, both for their cultural and ecological impact. What would the loss of such a prominent tree mean for the species and people who rely on them?

This question isn't just hypothetical, the threat to Oak trees has grown considerably. With existing & emerging pests and diseases, and the growing climate emergency, the time for action is now. Action Oak's mission is to lead the vital work needed to safeguard the future of our native Oak trees.

Action Oak is working to protect the UK's native Oak trees by leading the vital research taking place to protect these iconic trees. Our guiding principles help us to fulfil these goals.

Guiding principles

- Protect our native Oaks for future generations.
- Be collaborative, innovative, and committed to our research.
- Share findings with practitioners to grow healthier, stronger trees.
- Raise public awareness and appreciation of our native Oaks.
- Advocating for their significant role in our landscapes as a habitat for wildlife.

The Action Oak vision works towards:
A UK where native Oak trees are protected and flourish, both now and in the future.

Oak trees need urgent help to safeguard their future.

Support our mission

Donate | Monitor | Ambassadorship

To find out more, visit: **www.actionoak.org**

Connect with us

Twitter
@ActionOak

Facebook
facebook.com/ActionOak

Instagram
@actionoak

ActionOak
PROTECTING OUR OAK TREES