ActionOak
PROTECTING OUR OAK TREES

Action Oak Report 2021

Contents

Front cover photo: John Glover IGPOTY

Foreword

by Lord Gardiner of Kimble

The Oak tree is perhaps our most iconic and loved tree in the UK, it has a unique place in the landscape and forms an integral part in our shared history. From trees in the local park to the Atlantic Oak woods, they are a quintessential part of our national identity and hold a very special place in all our hearts.

Our wonderful Oaks are, however, under threat from climate change and pests and diseases and we need to act now to protect these glorious trees for the future. It is vital that we all realise the importance of our nation's tree health and the vital need for research to help address the threats they face. Pests and diseases are challenging our Oak trees' very survival. Oak processionary moth, Acute Oak Decline, root-attacking species of honey fungus and powdery mildews are all present in the UK today and weakening or killing our majestic Oak trees with the bacterial disease, *Xylella fastidiosa*, an increasing threat as it moves its way across Europe towards the UK.

The vital research that is being carried out by Action Oak, with many institutions around the UK, is enabling a deeper understanding of both the threats facing our Oaks as well as what can be done to protect them for future generations. It is essential that our Action Oak researchers collaborate to share knowledge and learnings from their work, to pool and grow our collective understanding about our Oaks.

From working to discover the mechanisms involved in Acute Oak Decline and how a tree's immune system might be strengthened via its microbiome, to what drives acorn production and how our Oak trees might adapt to a changing climate, the researchers are working to discover more about the UK's best loved tree. By monitoring the Oaks around the country we are also building a comprehensive picture of Oak health and the threats they face. The research is also paving the way for better management of our iconic Oaks, ensuring the findings are translated into useful guidance for tree and woodland managers, to provide them with the practical tools to manage our existing Oak trees, to secure them for future generations.

Please do read this update and support the work of Action Oak.

Photo: Ben Hirst IGPOTY

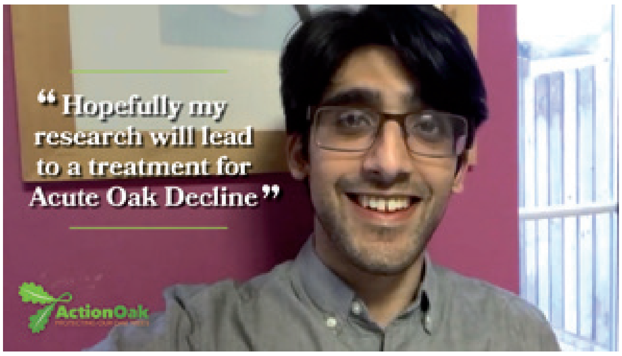
Introduction

by Geraint Richards, Chair, Action Oak

On behalf of the Action Oak Steering Committee I would like to take this opportunity to thank everyone who is involved in Action Oak. The publication of the first 'Annual Report' is an important milestone for Action Oak, highlighting the vital research that is being undertaken to preserve the health of the U.K.'s most important tree, at a time when the threats to it are many and varied. Alongside the researchers, there are many others who are supporting Action Oak and the further development and strengthening of this community, focussed on our native Oak trees, has become a key aim of the initiative. I would particularly like to thank Lord Gardiner who has been a keen supporter of Action Oak since its inception and remains, in his new role as Senior Deputy Speaker of the House of Lords, an Ambassador for Action Oak.

Please do continue to support the valuable work of Action Oak.

This comes with my thanks and best wishes



Action Oak research update

by Jasen Finch, Aberystwyth University

Recent work by Aberystwyth University and Forest Research, funded by Woodland Heritage, has developed a novel tool for assessing the visual health and decline status of Oak stands. The tool determines severity and type of Oak decline using a series of simple observations and measurements describing the Oak trees' size, stature, crown health and stem symptoms. Trees can then be assigned a decline index score on a continuous scale between 0 and 1. This method has proven useful for allowing researchers to objectively describe the spectrum of Oak decline in study trees, and it also has great potential as a management tool guiding thinning operations and management interventions. The methodology for this tool is outlined in a recently published open-access article in the Forest Ecology and Management journal, titled "Index measures for Oak decline severity using phenotypic descriptors". The article is available at https://doi.org/10.1016/j.foreco.2021.118948. An interactive web application accompanying the article is also available at https://jasenfinch.shinyapps.io/decliner/.

The Aberystwyth University team consisting of Dr Jasen Finch, Dr Manfred Beckmann and Professor John Draper, have been investigating the chemical composition of living Oak wood using a technique known as metabolomics. It is hoped that this will identify chemical changes that take place when trees transition from healthy to a diseased condition and visa versa if they recover. Recent findings indicate that the levels of sugars found within these tissues could predict the likelihood that a tree suffering from Acute Oak Decline (AOD) could transition into remission. In time, these trees may then recover from the syndrome. The sugars present in the trunks of Oaks are key indicators of the general health and vigour of a tree. This exciting finding could enable the development of tests, suitable for use in the field, that could provide vital information in guiding forest management and intervention strategies.

The Aberystwyth team will be applying these methods further as part of the recently funded FUTURE OAK and Bac-Stop projects. For the FUTURE OAK project led by Bangor University, they will be relating changes in wood chemistry, to differences in the microbial communities present in Oak trees affected by AOD. This will be investigated at the landscape scale with trees sampled from across Britain. In the Bac-Stop project, which is led by Forest Research, they will be analysing changes in wood chemistry during the early stages of AOD establishment. This will be part of a holistic experiment, being conducted under controlled field conditions. More information can be found about these projects from the respective web pages below as well as later in this Report.

FUTURE-OAK
www.future-oak.com

Bac-Stop
www.bacterialplantdiseases.uk/bac-stop.html

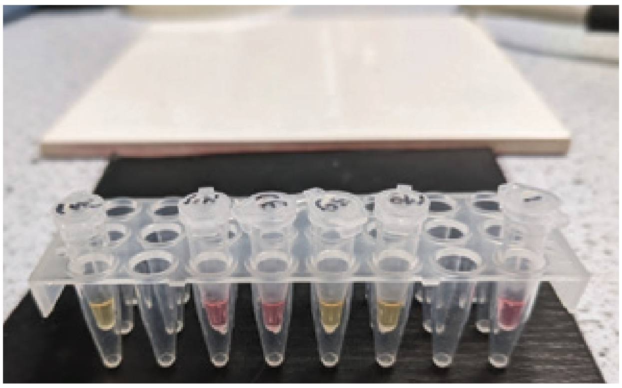
Investigation of the soil rhizosphere role in AOD

by Daniel Maddock (PhD student, University of the West of England, Bristol)

With so many factors contributing to the development and spread of AOD it's of no surprise that soil, the physical support system and its associated biotic and abiotic factors are of interest. The rhizosphere, the region of soil surrounding roots that is seen to be influenced by plant secretions and their associated microorganisms, was first proposed in 1904 by Lorenz Hiltner. It is an essential feature of plant health, being the first point of contact between microbial communities found in soil and trees. Soil microbiota in the rhizosphere are well known for their plant growth promoting ability, mainly by mobilising nutrients such as nitrogen and potassium or by acting antagonistically towards pathogenic microorganisms. However, it is also a point where phytopathogens can negatively affect plants and move into the internal sections to have more negative effects.

Daniel Maddock (PhD student, UWE)

I am a second year PhD student at the University of West England (UWE), jointly funded by Woodland Heritage and UWE. My project aims to highlight the differences in the soil bacterial community of healthy versus diseased Oak trees. Back in October I took a trip to Hatchlands Park in Surrey and collected eighty rhizosphere samples (roots and all) from AOD symptomatic and non-symptomatic trees in a paired model system.



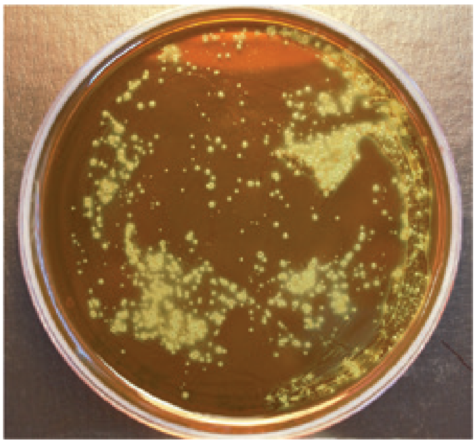
Collection of Oak root samples

The roots in these samples have since been identified as belonging to Oak, using a novel method developed at Forest Research to ensure that consequent results gained from these samples are relevant to the field of study. This is done by amplifying Oak specific genes from roots causing a colour change reaction from red to yellow.

I have extracted the DNA from all bacteria present in the soil for synthetic long read 16S rRNA sequencing. This will allow for the identification and quantification of bacteria present in the rhizosphere. These results will serve two purposes, firstly allowing the identification of bacterial groups of interest (based either on their function or prevalence in samples). The bacteria of interest will then undergo specific amplicon-based sequencing of conserved housekeeping genes to allow concise species level taxonomic assignment of members of the rhizosphere.

The second purpose will be to begin to identify differences between AOD positive Oaks' and healthy Oak trees' root microbiome. These differences could be in the presence of antagonistic pathogens in the AOD rhizosphere, or the lack of positive plant growth promoting bacteria present around the roots of healthy Oaks. The overall aim is to identify on a species-specific scale what role soil microorganisms play in the development of the disease.

Alongside the molecular genomics-based approach, is a more traditional microbiological culturing plan. Currently, I am using selective recovery broths to isolate members of the Enterobacteriaceae from the Oak rhizosphere. As we have no prior information about the groups of interest present in these samples, it made sense to focus on the Order of bacteria that are already known to play an important role in AOD. *Brenneria goodwinii*, *Gibbsiella quercinecans*, the key members of the AOD lesion microbiome and



Single isolate

Rahnella victoriana and *Lonsdalea britannica*, potentially important secondary members of the lesion microbiome all belong to the Order Enterobacterales. Interestingly these bacteria have unknown origins as they do not appear to be endophytes present in core samples from healthy Oaks.

As such it has been suggested that soil could function as a reservoir for infection, meaning once the tree is weakened by environmental 'predisposition factors' pathogenic bacteria can invade the inside of the tree via cracks in the roots' system, while the tree's innate immune system is less capable of responding to negative stimuli. High resolution melt analysis of samples in recovery broth has since allowed the identification of *Rahnella* from both healthy and diseased soil as well as the recovery of *Gibbsiella quercinecans* from one AOD positive root samples. However, further work is needed to identify consistent patterns in the presence of these pathogens from soil.

Singles isolates are also being recovered from these samples, using a range of dilutions and different incubation conditions, with the aim of identifying new undescribed species of bacteria (and getting some wonderful pictures). These can then be used to further inform the taxonomic description of related species. This will also be done for the groups of interest informed by the 16S rRNA sequencing, to aid the amplicon sequencing.

Photo: Marie Davey IGPOTY

Whole genome sequencing of *Quercus robur* and *Quercus petraea*

by Gabriele Nocchi and Louise Gathercole, Royal Botanic Gardens, Kew

Gabriele Nocchi and Louise Gathercole are PhD students funded under the Action Oak initiative by a Defra grant to the Royal Botanical Gardens Kew. They are based in the lab group of Prof Richard Buggs at Kew and at Queen Mary University London. Gabriele and Louise have been working on the whole genome sequences (all of the DNA in a sample) of over 400 *Quercus robur* and *Quercus petraea* from five UK sites, four of which have trees with symptoms of Acute Oak Decline.

Leaf and twig samples were collected Autumn 2017 by the Forest Research Technical Services Unit. The samples come from sites monitored by Forest Research and their partners over a number of years for Acute Oak Decline symptoms, crown condition, age and size.

DNA was extracted from the samples at RBG Kew and the samples were sent off to be sequenced by Novogene in Hong Kong, generating over 10 terabytes of data. Gabriele mapped the data to a high-quality reference genome assembled by scientists at INRA in France. The oak genome is 750 million DNA letters long, and Gabriele found millions of points on the genome that vary among English oak trees.

Gabriele's initial aim was to assess whether differences between the genomes of oak trees cause differences in their susceptibility to AOD symptoms. This would help to find out whether it would be possible to breed oak trees with resistance to AOD. However, after several efforts, the sample size was deemed too small for this type of analysis with such a complex decline disorder. In the future, the team hopes to sequence more samples and try the analysis again.

Gabriele's research then focused on oak genomics and hybridization and his aim was to uncover the genetic components which determine *Q. robur* and *Q. petraea* diversity as well as to identify ecologically important genes. The project also investigated the post-glacial routes by which oak trees colonised Britain using DNA from chloroplasts. Gabriele's research has been published and is available at: https://doi.org/10.1002/ppp3.10229

Photo: Clare Park IGPOTY

Louise's first project is looking at the genomic sequences that do not belong to the oak tree. When DNA from a leaf sample is extracted and sequenced, not only is the DNA of the plant sequenced, but also many of the microbial life forms that are living on and in the leaf. Louise is using bioinformatic tools to see if there is genomic evidence of the bacteria previously found in stem bleeds, on the leaves and whether there are differences between healthy and unhealthy trees in the microbiome. So far there is some limited evidence for the existence of AOD bacteria on the leaves in all of the woodlands in the study and on healthy as well as symptomatic trees.

Louise is now to analysing some new genomic data which is was sequenced earlier this year by Novogene. The oak leaf samples come from 60 sites across Britain that are part of the Forest Condition Survey funded by The Forestry Commission and the sequencing is funded by Defra Future Proofing Plant Health. Louise will be building on Gabriele's work on oak diversity. She will also be looking into genetic differences in the oak trees in different parts of the country to see if they are linked to the environment. This may shed light on the genotypes that are likely to be best adapted to a changing climate.

For both projects Louise and Gabriele work with Nathan Brown of Woodland Heritage and Forest Research who manages the survey data for both projects.

BIFoR overview

University of Birmingham, Institute of Forest Research

(L to R); Carolina Mayoral, Estrella Luna-Diez, Rosa Sanchez Lucas and Isabel Okeke

The Birmingham Institute of Forest Research, BIFoR, was established in 2013 and carries out cross-disciplinary research on trees and forests and associated features. Several new staff (Prof. Robert Jackson, Dr Florian Busch, Dr Megan McDonald, Dr Laura Graham, Dr Adriane Esquivel Muelbert, Dr Mojgan Rabiey) have been recruited to key positions. Together, over 40 staff now work on projects studying the impacts of climate change, and pests and pathogens, on trees and woodlands. This has already led to the award of a number of cutting edge government-funded research projects, including QUINTUS (funded by the Natural Environment Research Council, NERC), FACE-Underground (NERC), DiRTs (NERC/NSF - National Science Foundation) and a *Pseudomonas-Prunus* study. Several of these make use of the linchpin Free Air Carbon Dioxide Enrichment (FACE) facility, located in Mill Haft woodland in Staffordshire and include Oak research. BIFoR FACE has been described in the literature (Hart et al., 2019, doi:10.1111/gcb.14786); a further description, focusing on plant-water interactions, is under review (MacKenzie et al., 2020; https://doi.org/10.22541/au.160157598.86879557)

Despite 2020's challenges due to the COVID pandemic, colleagues in BIFoR have implemented robust health and safety procedures to ensure safe working practice, especially in the outdoor sites. The FACE facility worked throughout the 2020 growing season and fumigation with CO2 was finished on time at the end of October. It has been a good year for forest productivity, with healthy canopies observed via the 'PhenoCam' (https://phenocam.sr.unh.edu/webcam/browse/millhaft/) and plenty of acorns raining down on the researchers and equipment! The first BIFoR FACE PhD thesis has been submitted; Liam Crowley will defend his work on "Are insects key drivers of change in woodland systems under climate change?" early in 2021.

The first paper based on PhD work using the FACE facility has been submitted (Gardner et al., Is photosynthetic enhancement sustained through three years of elevated CO2 exposure in 175-year old *Quercus robur*?).

Funding from the JABBS Foundation has enabled two postdoctoral research fellows, Dr Rosa Sanchez-Lucas (working with Dr Estrella Luna-Diez) and Dr Thomas Welch (working with Dr Graeme Kettles), to carry out research projects examining defence mechanisms in Oak that can help to protect the tree from pathogen infection. One aim has been to establish and optimise protocols for performing an Oak seedling diversity panel screen against the Acute Oak Decline (AOD) bacterial complex and the Oak powdery mildew (PM) fungus. A new protocol for generating stem infections of Oak seedlings with AOD bacteria and a medium-throughput image analysis platform for the quantification of foliar disease symptoms induced by the PM fungus on Oak leaves has been developed. These will allow experiments to be done that can identify two main components of defence: priming of resistance; and identification of resistance alleles that help fight off infections by pathogens. Publications are in-train (Sanchez-Lucas et al., Disentangling the effect of elevated CO2 in growth and resistance mechanisms against powdery mildew in Oak seedlings; Mayoral et al., Elevated CO2 does not improve the regeneration of a mature Oak woodland subjected to biotic stress).

BIFoR continues to support the Action Oak initiative including via a very well attended AO session at the BIFoR conference in January 2020. Despite the best efforts of the virus, we were able to continue the conference in 2021 via a virtual session, some of the talks and all of the posters are available on the 'BIFoR fifth Annual Meeting' website.

Summary of the Bac-Stop project research results covering the period September 2020 - April 2021

by Sandra Denman, Forest Research

Last year, our group was fortunate to win a Biotechnology and Biological Sciences Research Council (BBSRC) grant (£2.5M – 80% BBSRC funded) for a project called Bac-Stop, which runs for 3 years as part of the Bacterial Plant Diseases Programme (BPD). The main aim of the Bac-Stop project is to address practical unresolved and contentious issues on various aspects of AOD, and deliver the evidence required to develop solutions so that pragmatic management of the problem can be implemented. We thank Action Oak for its strong support of our project and here, give a brief overview of the scope of the project and highlight a few recent results.

The Bac-Stop project has four main research areas (work packages). **WP1** addresses the question of whether or not the native jewel beetle, *Agrilus biguttatus*, is essential to the development of AOD. Research evidence shows that *A. biguttatus* is present in almost every case of AOD, the geographical distribution of the beetle and AOD correspond and when co-inoculated into logs, the number and abundance of damaging bacterial genes responsible for tissue necrosis in AOD are increased 10 fold. However, there is still a high level of contention about the role the beetle plays in AOD. Therefore, we set out to produce definitive evidence that would support or reject the hypothesis that the beetle is essential to the development of AOD. To achieve this, we are investigating whether the AOD bacteria occur in or on the beetles, and if the beetles can transfer the AOD bacteria into trees (Figure 1). To date we have detected the AOD bacteria in newly emerging adult beetles (Table 1). Interestingly in newly emerged beetles, bacterial species diversity is low. When beetles were fed natural forest leaves the AOD bacteria were detected at moderate levels in all body parts, compared with low levels in beetles that had fed on Oak leaves from laboratory reared plants. However, when laboratory leaves were dipped into bacterial suspensions prior to beetle feeding, very high levels of the bacteria were detected. Even though molecular tools detected the bacteria, we failed to culture them, but attribute this to methodology and have now improved culture techniques and will test this again in the coming summer.

We are also investigating whether substances produced by the beetles and their larvae can trigger upregulation of

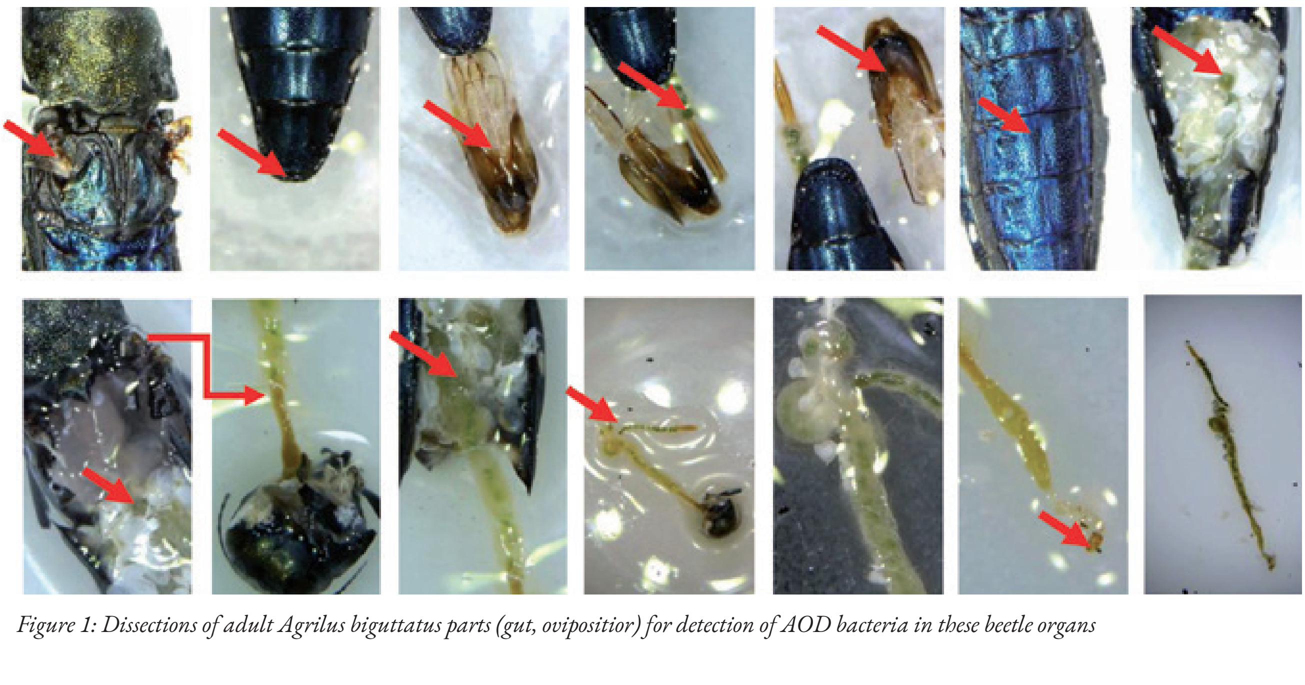
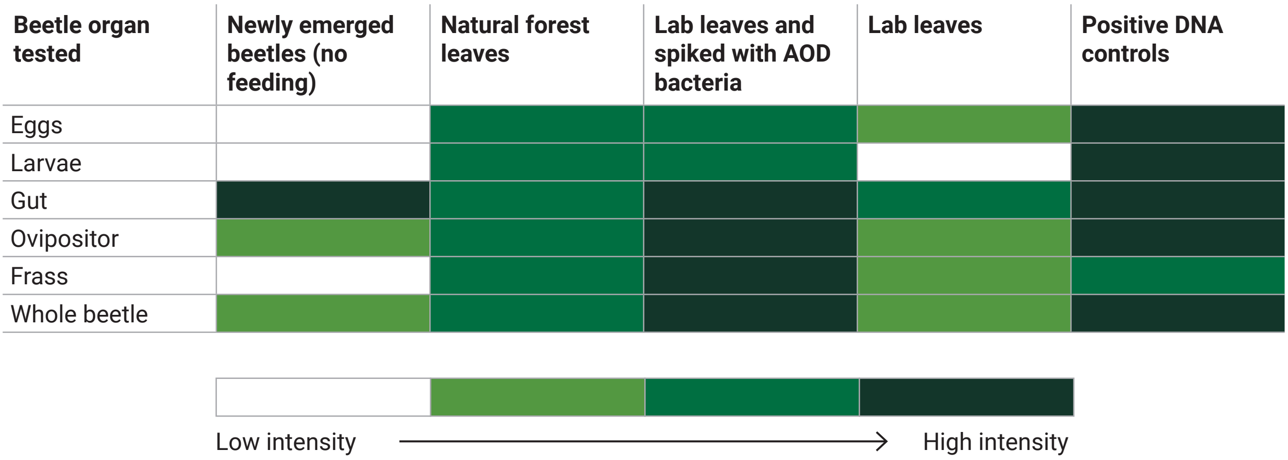


Figure 1: Dissections of adult Agrilus biguttatus parts (gut, ovipositor) for detection of AOD bacteria in these beetle organs

Table 1: Heatmap showing detection of AOD bacteria in various beetle organs following beetle feeding on leaves with a range of treatments



damaging genes in the AOD bacteria, and whether odours produced by the bacteria can act as attractants for the beetles. Using chemical ecology, results show that females carrying eggs are more strongly attracted to odours of all three AOD bacteria combined than individual sp. or the control medium. The chemical constituents of the odours are being analysed.

In **WP2** a field trial is being set up to test the hypothesis that sustained drought causes biochemical, physiological, metabolomic and microbiome changes to Oak, rendering them susceptible to AOD. This supports the idea that predisposition that weakens trees is essential for AOD to develop, and drought is a major driver of predisposition. The field trial is one of only a very few such trials in the world. Drought shelters have been constructed (Figure 2) and wireless soil monitoring dataloggers and TreeTalker instruments will be installed soon. The TreeTalkers are pioneering, tree physiology continuous measurement instruments that have recently been developed. In the summer, beetle eggs and AOD bacteria will be inoculated into control, droughted or nutrient deprived trees.

In **WP3** we are investigating managers' and public's attitudes to Oak, AOD and its management, and monitoring Oak health. We have analysed stakeholder composition, created a sampling strategy and developed a protocol of questions that we will use in interviews. We also recently launched an online platform for data gathering called 'Odes 2 Oaks' in which we are asking people to provide prose, poems, photos of what Oaks mean to them. This will contribute to an understanding of the emotional and sensory connections to Oaks that many people have. We are also investigating using our citizen scientist network to examine the type and distribution of predisposition factors evident through monitoring sentinel trees and are working with the

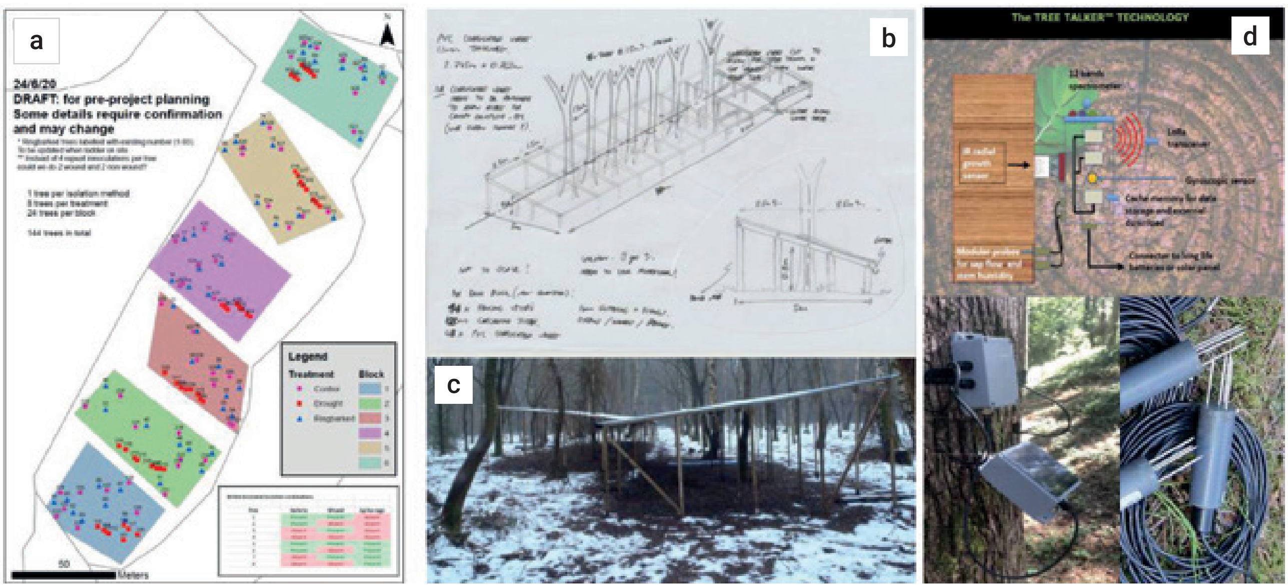


Figure 2: Experiment layout (a) construction plans of drought shelter (b) drought shelters constructed in the field (c) and soil and tree continuous monitoring equipment (d)

Figure 3: Images of the online platform for people to express their connections to Oak trees

Observatree network and beyond to foster greater awareness of Oak health and provide individuals and groups with the tools to detect change in tree health and record it.

Finally, in **WP4** we are assessing whether various bacterial spp. could be the cause of unresolved tree diseases in the UK, and whether the AOD bacteria could be involved. Tree Health Officers, FR pathologists and citizen science volunteers collect swabs (Figure 4) from bleeding cankers on broadleaf trees (beech, lime, birch, sweet chestnut, Oak), which are tested for the AOD bacteria using multiplex PCR and bacteria are isolated and identified. So far, we have identified the AOD bacteria *Rahnella victoriana* on other broad leaf tree species and *Brenneria goodwinii* from Oak. However, of particular interest are new species of *Brenneria* and *Erwinia* isolated from bleeding cankers of lime trees (*Tilia sp.*). These isolates need to be taxonomically classified and their pathogenicity tested in log trials.

BAC-STOP
STOP BAD BACTERIA ON OAK

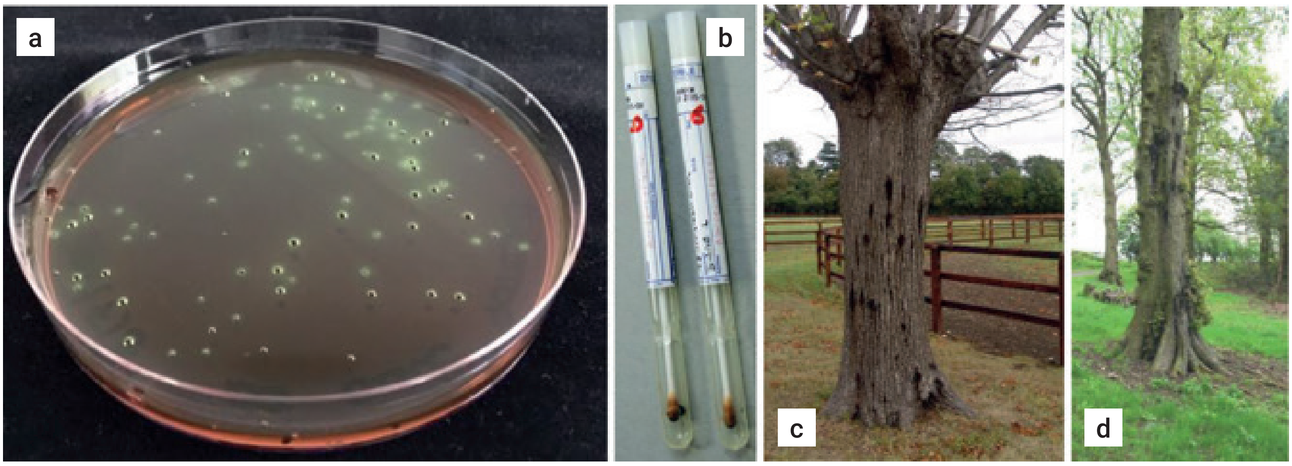


Figure 4: (a) Petri dish with bacterial colonies on it. (b) Swabs that have been used to swab weeping lesions on trees. (c+d) Lime trees (Tilia sp.) with weeping lesions of unknown cause but potentially Erwinia sp(p) could be the cause of this disease

Oak monitoring

by Nathan Brown, Woodland Heritage

In this year of all years, local green spaces have taken on new meaning, as places for momentary escape and respite. By exploring these spaces, we have an increased awareness of their changing appearance through the seasons and connections that were previously overlooked have become all the more visible. Within this heightened awareness lays great potential to contribute to research efforts designed to understand long-term environmental change. By taking note of seasonal and annual changes patterns and trends can be revealed. In 2019, Action Oak (in collaboration with Defra and the Forestry Commission) set out to re-establish a long-term monitoring network, which began recording Oak health in the 1980s. The health of Oak trees can be recorded by measuring their growth and by estimating the number of leaves in their crown. The volume of the crown and the density of leaves within it give an estimate of how much energy is available to the tree, as trees with more leaves can photosynthesise more and will produce more sugar, which can be used for growth and to fight off pests and diseases. It is hoped that by understanding the changing state of Oak health we can better plan for future change and spot trends early. Sites with declining health can be investigated to reveal the drivers behind this change and enable mitigation efforts to be developed and deployed in good time.

The Oak monitoring sites were established as part of a multi-species Europe wide network which was monitored between 1987 and 2007. We now have a further data from 2019 and 2020 at all the remaining sites in England, Scotland and Wales. In 2021, monitoring has continued and work has begun in order to expand the network. Work is underway to develop the methods for site owners, managers and volunteers to observe additional sentinel trees at sites they visit frequently. This work is part of the Bac-Stop project led by Sandra Denman (Forest Research) and funded by the BBSRC, NERC, Defra and the Scottish Government. There have been a number of challenges to overcome to ensure training could proceed as planned in 2021, but we have been lucky to work with Peter Crow and Suzanne Sancisi-Frey from the Observatree programme, who have lent their expertise to provide options for both in-person and online training and they have even devised methods for virtual tree assessments to support the work.

Crown thinning due to AOD

Photo © Forestry Commission

Oak boring beetle Agrilus biguttatus

FUTURE OAK project launched to safeguard our iconic Oaks

by James McDonald, Bangor University

Climate change causes an increase in diseases affecting our iconic Oak trees, but a pioneering project is to investigate the role of beneficial microbes in fighting diseases that affect our native Oak trees.

The FUTURE OAK project, comprising scientists at Bangor University, Aberystwyth University, Forest Research and Sylva Foundation, will study how Oak microbiomes are affected by environmental change and disease.

The UK is home to around 170 million Oak trees, and more ancient Oak trees than the rest of Europe combined. Native Oak support over 2000 species of insects, birds, mammals, and fungi, but climate change, human activity, and outbreaks of tree disease are affecting the health of our forests. Acute Oak Decline (or AOD) poses a significant threat to our native Oak trees. Trees with AOD are weakened by environmental stresses, like drought, and several different bacteria cause the inner bark tissue to rot. Bark-boring beetles also feed on the inner bark of weakened trees, further increasing bacterial activity. Eventually, the outer bark cracks, releasing fluid from the rotting inner tissue and causing the distinctive stem 'bleeds' that are observed on trees affected by AOD.

Much like humans are colonised by trillions of microorganisms, trees also live in close association with bacteria, fungi, and other microorganisms. Different communities of microorganisms are associated with tree stems, roots, and foliage, where they play important roles in acquiring nutrients for the plant, regulating plant immunity, and suppressing pathogen attack. These collections of microorganisms, their interactions, and the parts of the host plant where they are active is called the 'microbiome'. Beneficial microbes in a tree's microbiome are essential for fighting diseases.

Using DNA sequencing technology and by culturing microorganisms from healthy Oak trees in the laboratory, the FUTURE OAK project will analyse the microbiome of hundreds of native Oaks across Britain to understand which microbes promote health and fight diseases. By comparing the composition of the microbiome with tree health status, and by testing isolated microorganisms for their ability to suppress Oak pathogens, we will identify beneficial members of the Oak microbiome community. This will help us to develop biocontrol treatments for the Oak microbiome, to promote healthier trees and suppress the symptoms of AOD. Introducing the microbiome to our thinking about tree and forest health presents a distinct challenge. Consequently, a major component of FUTURE OAK will be to work with land managers to explore their understandings of the role of the microbiome in plant health. In particular, the team will be exploring perceptions and views on the potential to use 'engineered' microbiome products to prevent and treat Oak disease.

The research is supported by £1.3M of funding from the Bacterial Plant Diseases programme funded by the Biotechnology and Biological Sciences Research Council (BBSRC), Natural Environment Research Council (NERC), Defra and Scottish Government and is also supported by Action Oak.

For more information about the project, please visit: www.future-oak.com), Twitter profile (@FutureOak_), or email James McDonald (j.mcdonald@bangor.ac.uk).

FUTURE OAK animation

A mature Oak tree in North Wales

Enhanced acorn production to regenerate native Oak woodlands in the UK

by Rory McClory, Future Trees Trust

My name is Ryan McClory. Last year, I began a PhD project in partnership with the Future Trees Trust (part-funded by Action Oak) and I have been given the opportunity to tell you a little about myself and what my research entails.

From Bath, I moved to Plymouth, where I completed an undergraduate degree in Conservation Biology, and then to Stockholm, where I completed a postgraduate degree in Ecology and Biodiversity. At Stockholm University, I studied the effects of germination timing on attack by pathogens and herbivores for Oak seedlings. Last September, I began a PhD in partnership with the Future Trees Trust. The PhD is based in the school of Agricultural Policy and Development at the University of Reading and is entitled "Drivers of Oak masting in the UK." As part of this project, I intend to aid the regeneration of native Oak woodlands in the UK via enhanced acorn production.

In 2020, acorns were produced in large numbers, synchronously, across the UK. This phenomenon, known as "masting," occurs every 4-7 years in British Oaks and is a common reproductive strategy for several taxa of perennial plants. Masting floods the ecosystem with a sudden abundance of resources for consumers, influencing flora and fauna across trophic levels. While the mechanisms of masting remain under discussion, the phenomenon is widely thought to provide an evolutionary advantage for Oak species. The evolutionary advantage of masting can be explained using a microeconomics concept called "economies of scale," which states that the cost per individual product is reduced if many products are produced simultaneously – this is why Warburtons don't bake one loaf of bread at a time. This concept manifests among Oaks whereby seed predator species which use acorns as a food source have a reduced population size because, in most years, Oaks produce a limited acorn crop. Consequently, when Oaks produce a much larger crop, seed predators are overwhelmed by the supply. This means that, in mast years, although more acorns are eaten overall, the low population of seed predators increases individual acorns' chance of surviving to germination.

If masting is beneficial for Oak reproduction, it presents a challenge for Oak regeneration projects insofar as it makes a reliable source of acorns hard to find. Acorns are already problematic for seed providers due to their recalcitrant nature: they cannot be frozen or dried out without becoming inviable. In real terms, this means that reforestation projects which rely on demand for acorns being met by each year's crop are often forced to import acorns from outside the UK (usually, from continental Europe and the Netherlands). Outsourced acorns may be less well adapted for growth in the UK than British acorns, for example, by coming in to leaf early in the spring, which may result in frost damage and produce lower quality timber trees in the UK. Given that Oaks currently face additional pressures – air and soil pollution, unprecedented disease pressure, rising numbers of pests, and changing climatic patterns – the need for a consistent supply of British acorns is especially important.

To assist with guaranteeing a sustainable acorn crop in the UK, I will pose questions which incorporate several non-exclusive research areas:

(1) What are the environmental determinants of Oak masting at the UK-wide scale?
It is possible to use long-term data relating to acorn production to determine whether certain environmental factors act as cues for production in the UK. Analysing this data may offer a means of alerting seed providers in advance of mast years. Moreover, when compared with long-term environmental data, historical acorn production data may indicate whether climate change influences acorn production and how this might play out in coming years.

Photo: Simon Hadleigh-Sparks IGPOTY

(2) **How do the resource dynamics of individual trees affect acorn production?**
Determining how acorn crops are influenced by the internal resource dynamics of trees could help to pinpoint the most important nutrients for acorn production. This information could be used to direct resource manipulation measures which seed providers could use to promote a more consistent acorn crop in the UK.

(3) **What are the causal factors of flower or acorn abortions?**
Each year's acorn crop is determined by the successful fertilization of each tree's flowers and their subsequent maturation into acorns. However, we do not know at which stage in the process acorn production is halted during non-mast years. By determining when, among non-masting trees, acorn production is halted, researchers could facilitate the development of methods to prevent premature acorn abortion and stimulate masting artificially.

(4) **How does acorn quality differ between masting and non-masting trees?**
Poor acorn quality, including small acorns which are particularly vulnerable to desiccation, can hinder regeneration projects. If researchers could determine which conditions incur higher quality acorns, management strategies to increase the maturation of acorns into trees could be foreseen.

The story so far

For my research, I have access to Wytham Woods, an area of woodland which covers 1,000 acres and is a Site of Special Scientific Interest. Wytham was bequeathed to the University of Oxford in 1943 and, in 1962, the university decided that the woodland's primary purpose should be scientific research. Today, Wytham is one of the most studied woodlands in the world.

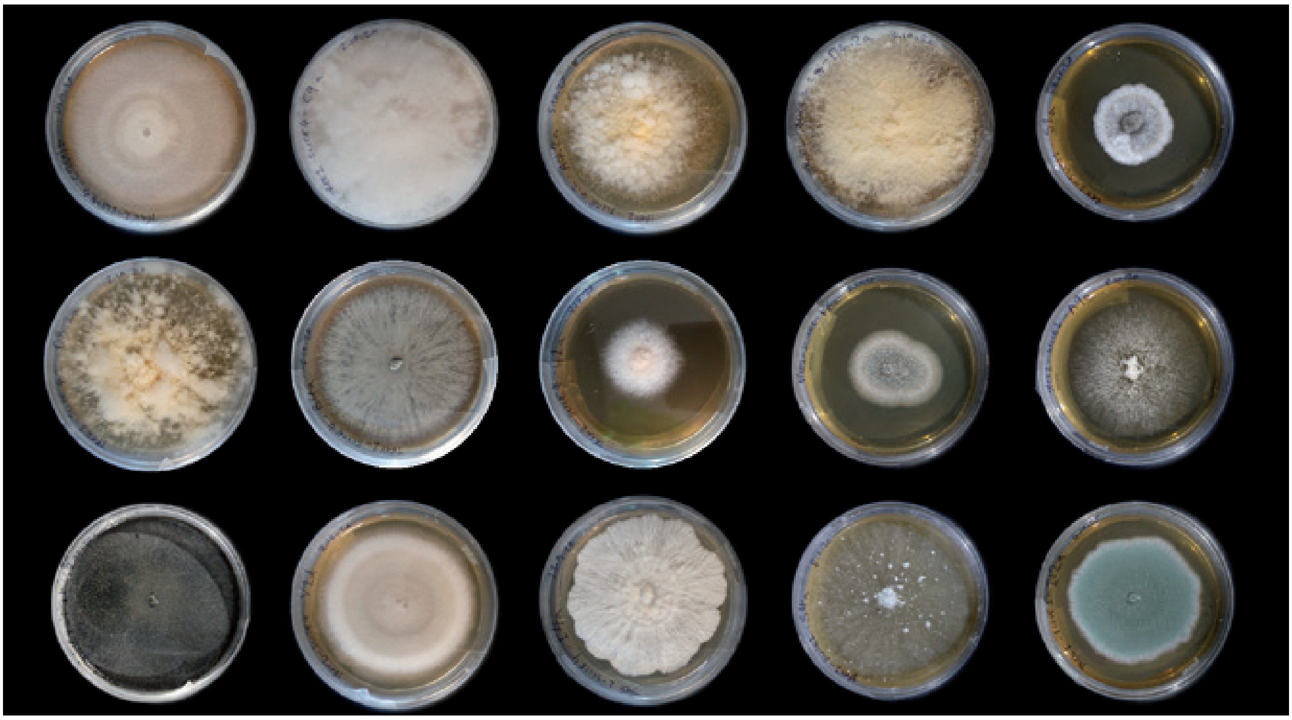
So far, I have selected 40 Oak trees across the site and attached small temperature and humidity monitors to each one, with a view to studying the environmental conditions which precede good acorn production. I have also collected around 3,800 acorns for use in germination experiments and to measure their moisture content, which will enable me to judge the variety of acorn quality in mast years.

The next stage of my research will involve gathering acorn production and meteorological data from across the UK. I will use this data to investigate how and under what conditions acorn production has changed since the mid-twentieth century and how it might function in the future. I will also construct litter traps around my experimental trees at Wytham to record premature acorn abortion and to collect mature acorns in non-mast years to test their quality.

My doctoral research in partnership with The Future Trees Trust is funded by several supporters: The Patsy Wood Trust; Action Oak; The Scottish Forestry Trust, The Mabel Cooper Charity; The D. G. Albright Charitable Trust.

Fungal heart-rot communities in British Oaks

by Richard Wright, Cardiff University



Cultures of fungal heart-rot

My research into the fungal heart-rot communities in British Oaks has been underway for a year now. Despite some of the most difficult restrictions, due to lockdowns and COVID, which have greatly delayed field work, there has been some good progress.

During the period in between lockdowns last year it was possible to collect the recently felled trunk of a 350 year old Oak, which was sectioned to produce 6 slices at regular height intervals. These slices were then sampled from by short drilled cores through the cut surface using a randomized grid method to select sampling points, all performed under sterile conditions. This produced over 500 initial cultures, eventually resulting in over 200 isolates which are made up of around 60 morphotypes (cultures that look the same). These morphotype cultures are now being DNA sequenced to reveal which species are present and this will then give an indication of the 3 dimensional fungal community structure once these names are assigned to the original sample points. The whole process has been successful so far and this sets a good standard for sampling from other trees in this manner. I hope to sample from 6-10 trees in this way over the next 2 years.

In response to having field work limited I have created the Standing Oak Tree Fungus Survey app, a community science project that I hope will provide some useful data to support sampling work. The survey aims to capture observation data of Oak trees that have fungal fruitbodies present, not just to obtain distribution data but to capture the condition of the trees and the fungi present. The app is aimed at field mycologists, arboriculturists and anyone who has an interest in fungi and trees. The app requires a little learning but the data required is quite straight forward for those with a little experience. You can find out more information and watch a short video at: www.foreverfungi.co.uk/oakfungisurvey/

Finding fungi on Oaks through observations and sampling only reveals part of the story, so to understand more I have setup a large interactions experiment, which will involve 14 key species of fungi found on Oak. In this experiment

Photo: Richard Wright

The Queen Elizabeth I Oak, Cowdray Estate, Sussex

hundreds of small cubes of Oak heartwood are colonized with one of each of the 14 species of fungi. This takes considerable time and preparation, around 2,500 blocks have been prepared and they will take 3-6 months to fully colonise. When they are ready, the blocks will be paired up so that one of each species is paired with all other species, with each pairing placed in its own separate environmentally controlled container. The fungi will then compete for territory, using physical and chemical attacks, which will lead to either deadlock or the replacement of one or other of the combatants. The results from this will give us insights into the succession and ecological niches that each species holds. It has been possible to carry out a few site visits over the last year and this has brought me up close with some amazing Oak trees. Observing Oak trees of all ages and the effects that fungi have on them is really valuable and helps to inform future lab work. Two particularly amazing Oaks on the Cowdray Estate, The Queen Elizabeth I Oak and The Maid-in-Waiting Oak, have been a real inspiration. The complete heart-rot of the former (pictured), which has now left only a thin band of functional sap wood, leaving a hollow that's nearly 5 meters across is quite astounding. As is the crossing hollows of the Maid-in-Waiting Oak, which have produced a deep pile of woody debris that is no doubt the home of many interesting invertebrates.

Over the next few months, now that field work can resume, I will be working with some of our masters students to carry out core sampling of Oak trees at a number of sites in Wales and England. This work utilizes an increment borer, which allows us to take 40cm core samples from living trees. These cores are then sampled from back at the lab and allow us to build a picture of the diversity of fungi in each tree. We're aiming to sample from around 60-80 trees in this way, of a range of ages, and this will give us insights into variations in diversity and also the succession that occurs as decay progresses.

As there is still very little known about the fungal communities of Oak heart-rot this project is really exciting as is often producing more questions than answers. After this year of sampling and experiments there should be some more interesting results to discuss.

Oak powdery mildew and tree health in the Celtic rainforest

by Octavia Hopwood, Aberystwyth University

Oak trees are synonymous with the British countryside, but they are currently facing a barrage of pressures that threaten their health and long-term survival, including climate change, pollutants and risk from pest and disease. The research we are conducting at Aberystwyth University is aimed at furthering our knowledge surrounding both the biotic and abiotic factors affecting tree health in Welsh Oak woodlands, with an emphasis on the mycobiome of these habitats. Within this project, tree health is explored in a holistic manner, characterized by a plant's ability to thrive and function as an ecosystem, rather than the traditional view that health is generally defined by organisms being free from disease. This project aims to explore how the pathogen 'Oak powdery mildew', a fungus that infects Oak leaves, fits in with the wider fungal community and how other fungal associations can affect overall tree health in relation to disease resistance.

Photo: Des Callaghan

Trilobata

The upland Oak woods in Wales are known colloquially as the Celtic Rainforests and are characterised by their westerly location, positioning within steep sided ravines and species such as *Quercus petraea* (Sessile Oak), *Betula pubescens* (Downy Birch) and *Corylus avellana* (Hazel). These habitats are of international interest due to their high fungal diversity, however there are very few detailed studies of these woodlands, particularly using DNA metabarcoding techniques. Aberystwyth University is undertaking a survey of 12 woodland sites across Wales to assess the distribution of fungal species and the severity of Oak powdery mildew. These sites include a range of 'Celtic rainforest' upland woodlands, as well as lowland Oak woodlands (*Q. Robur*) and other intermediate sites.

Research is currently being conducted into the methodology of DNA extraction and sequencing that will be used to analyse samples of leaf and soil material collected this year. Using data collected from previous trials, an evaluation of most suitable gene region for isolating and sequencing has been made. This will inform better decision making about the methodologies used for the project and enable better interpretation of the results.

Our main objective is to evaluate the distribution of the fungus that causes Oak powdery mildew in these forests, the main perpetrator being *Erysiphe alphitoides*. Using DNA metabarcoding, we are hoping to confirm the species of the fungus and assess its overall severity across the woodlands. Other species, such as *E. quercicola* and *E. hypophylla* also cause Oak powdery mildew and are currently seen across Europe, however these species have not yet been recorded in Wales (Chater & Woods, 2019).

In order to understand how the fungus interacts with other fungal species within the phyllosphere, we are aiming to gauge the level of species diversity that resides on and in Oak leaves. Our first hypothesis is that there is a unique 'fingerprint' of fungal communities living on the phyllosphere of Oak trees. To assess this, we have taken a pool of leaves from across our 12 sites and have sequenced them to review DNA abundance.

Following this we will then be able to determine if there is any correlation between Oak powdery mildew and other fungal species. Out of the plethora of fungal species that inhabit an Oak tree, some will be mutualistic, some saprophytic, and some parasitic or disease causing. Each individual species has its own niche, with some playing important roles in keeping the tree healthy, including sourcing water and nutrients. Since many fungal species form mutualistic relationships with trees (including

Photo: Mari Lloyd Photography

Celtic rainforest

mycorrhizal species), with some increasing resistance to disease, it is hypothesised that certain fungal species may increase Oak trees' ability to supress infection of *Erysiphe*. Using the species data collected, we're hoping to see either a lack of a specific fungal species in the presence of *Erysiphe*, or whether there is a correlation between increased visual outbreaks of Oak powdery mildew and other associated fungal species.

Factors affecting tree health may also affect fungal abundance. It is understood that changes in climatic and environmental conditions can significantly affect fungal populations, which in turn has implications upon species in symbiotic relationships. For instance, milder climates can exacerbate instances of Oak powdery mildew.

Air and soil pollutants are other factors thought to affect the health of plants, and increasing nitrogen is one such cause. Nitrogen pollution from industry and farming can affect both the air quality and soil health, and thus the health of plants too. We are interested in understanding the relationship between increased atmospheric nitrogen and the diversity of fungal communities on Oak leaves in the same area. Furthermore, if fungal diversity shifts, we want to assess what affect this may have on infection rates of diseases such as Oak powdery mildew. It is expected that a combination of pressures upon the Oak trees themselves, in addition to the fungal communities that reside on them, could lead to a downward spiral of health. Although Oak powdery mildew doesn't generally lead to tree death on its own, it has been thought to have been a contributary component in Oak dieback.

We hope to assess several woodlands subjected to higher nitrogen levels to compare general fungal community structure within these woodlands with woodlands in areas of lower atmospheric nitrogen.

In addition to field sampling, sapling trials are also underway and will be assessing the effects of various conditions upon Oak powdery mildew infection and severity. Conditions simulated include drought, waterlogging, high humidity and artificial fertilisation. A control has been included, as well as a group infected with Oak powdery mildew. Although these trials are still in the early stages of development, data collected may help to predict how various climatic conditions alters the prevalence of Oak powdery mildew and help to steer the project.

References

Chater, A. O. and Woods, R. G. 2019. *The Powdery Mildews (Erysiphales) of Wales: An Identification Guide and Census Catalogue*. Private Publisher. 50 p. ISBN: 9780956575036

Oak genetics and invasive pest biogeography

At Aberystwyth University we are using genetic and spatial modelling tools to guide future woodland management

by Rebekah Bristow, Aberystwyth University

Background

Increased global temperatures because of climate change, is not only exacerbating the spread of invasive pests by providing increased areas of suitable habitat, but also proving to be a major driving force in the alteration of species' genetic backgrounds through processes such as local adaptation. The current study at Aberystwyth University is investigating the vulnerability of Sessile Oak through the effects of climate change at both organismal (i.e., the genetic background of Oak) and species (i.e., distribution of invasive pests) level to help support and inform forest management.

Genetics

Microsatellite markers will assess gene flow and genetic differentiation in Oak individuals belonging to ancient semi natural woodlands in Wales and the wider UK. Since extensive hybridization occurs between the two *Quercus spp. Q. petraea* and *Q. robur*, alongside genetic markers, a morphological hybrid index has been calculated to delineate between *Quercus spp*. Individuals morphological characteristics have been scored and classified as *Q. petraea*, *Q. robur* or a hybrid. Both hybrid status, and valuable information in terms of gene flow and genetic structuring will be revealed, enabling a re-examination of the current seed zonation mapped for our native Oak. The use of pioneering technology, like next generation sequencing, offers a unique opportunity to investigate key questions such as, if there are any genes present in this keystone forest tree species which show signatures of selection across the UK seed zonation for Oak, and further, what metabolic pathways these genes are associated with (i.e., disease and pest resistance). Microsatellite analysis is due to commence in May 2021, whilst whole genome sequencing will commence in early autumn. Datasets will be analyzed in winter.

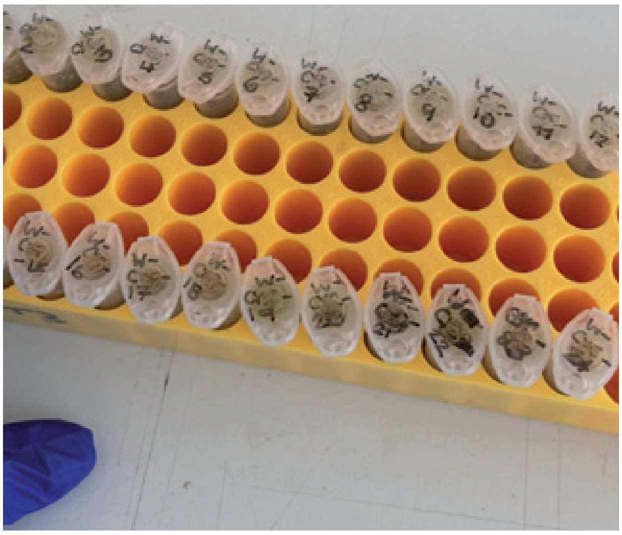
Species Distribution Modelling

The current and potential future habitat suitability of a selection of invasive Oak pests, including the Oak Processionary Moth (*Thaumetopoea processionea*) and the Common Froghopper (*Philaenus spumarius*), which vectors the devastating bacterium *Xylella fastidiosa spp.* multiplex, will be modelled under the different climate scenarios published in the sixth assessment report of the IPCC (Intergovernmental Panel on Climate Change). Biologically relevant predictors (i.e., dispersal capabilities) of pest species will be incorporated into building models to accurately forecast their dispersal and spread across Europe and the UK under future climate change. The project is currently building its modelling framework and preliminary map outputs will be produced by summer 2021.

Atlantic Oak woodland Coed Felinrhyd

Application and Importance

Improving understanding of our native Oak genetics is now paramount if we are to understand, predict, and attempt to manage its response to climate change and future pathogens



DNA extraction process – epitubes containing individuals DNA

and pest invasions. In addition, utilization of spatial modeling tools for pest risk mapping, enables effective early responses to pest outbreaks by highlighting high-risk areas which require cautious surveillance.

Support

- KESS 2
- Woodland Trust
- The James Hutton Institute & Aberystwyth University
- Forest Research - Alice Holt Forest

UK Sessile Oak Sample sites

Contact

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Photo: Simon Lea IGPOTY

Our vision

A UK where our native Oak trees are protected and flourish, both now and in the future.

Our mission

Our mission is to lead the vital work and research to protect our native Oak trees and safeguard their future.

Our values

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

About Action Oak

Without doubt, Oaks are the UK's most iconic trees. They are a fundamental part of our landscape and cultural heritage and remain crucial to this day, supporting both biodiversity and livelihoods.

But our Oak trees are under considerable – and growing – threat from pests, diseases, human intervention and the increasing climate emergency.

Action Oak is committed to protecting these incredible trees.

Action Oak is a national intiative made up of charities, government and private landowners who have come together to protect our Oaks for future generations.

Through carrying out vital research and monitoring, we can better understand the threats and devise real solutions, so that we can safeguard the survival of our Oaks for generations to come.

Our Oaks need you

Our mission is to lead the vital work and research to protect our native Oak trees and safeguard their future.

Our mission

Our Oak trees need urgent help to protect them for the future. You can support us by:

- Donating to Action Oak
- Helping us to monitor the health of our Oak trees
- Becoming an Ambassador for Action Oak and spreading the word about our work

Learn more about the threats to our Oaks and the work of Action Oak at: www.actionoak.org

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ActionOak
PROTECTING OUR OAK TREES