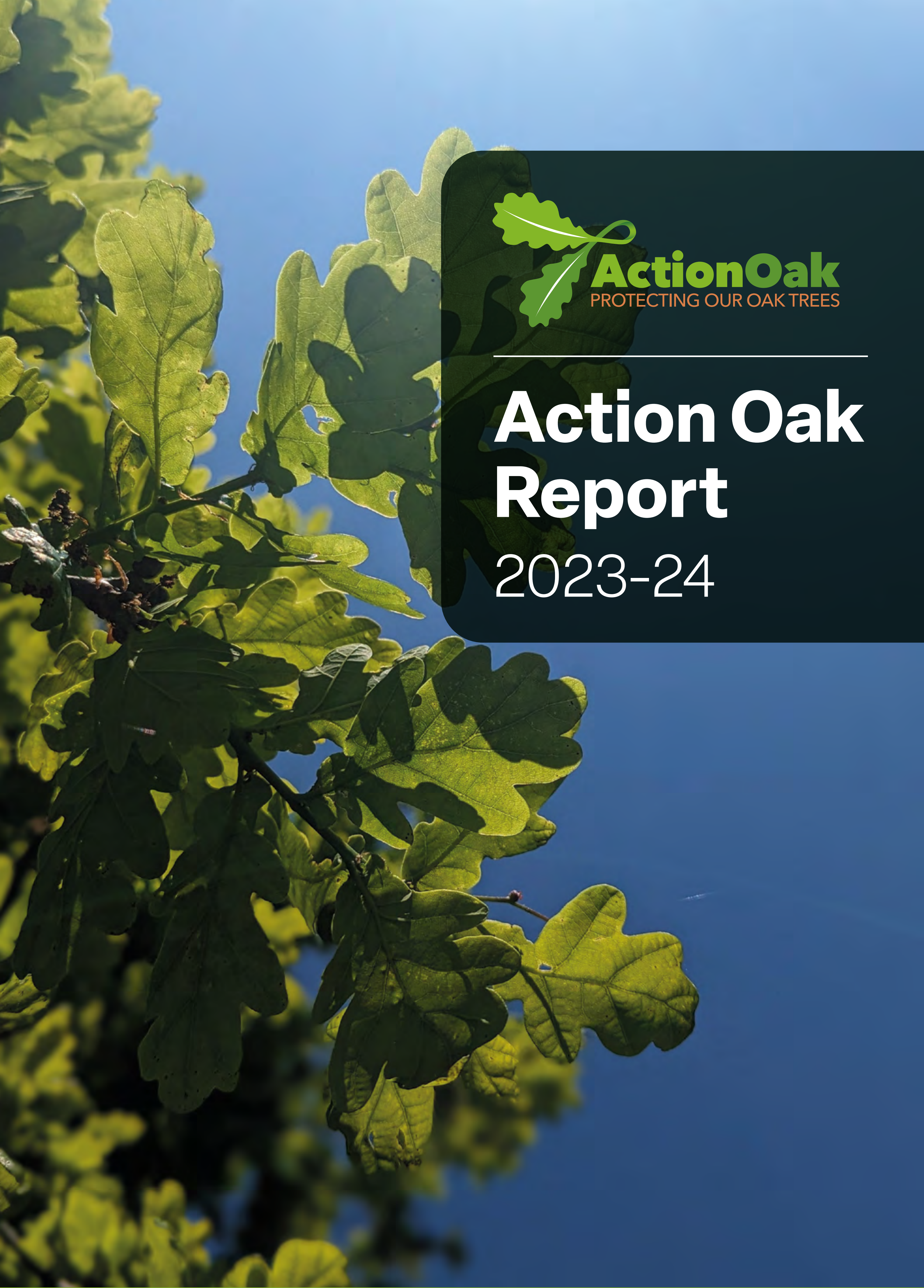

ActionOak
PROTECTING OUR OAK TREES

Action Oak Report

2023-24

Contents

Foreword

Huw Irranca-Davies, Cabinet Secretary for Climate Change and Rural Affairs, Welsh Government

Oaks are not only one of our most iconic trees in the UK, but they hold a profound place in our national psyche. Perhaps this is not surprising, as they are trees of great legends and mythology and we also have more ancient oaks than you can find across all our EU neighbours combined. Numbering around 170 million[1] oak trees in woodlands and 2.3 million trees outside of woodlands in the UK, these magnificent trees are deeply entrenched in our history, culture, economy and landscape. They are also fundamentally important in supporting wildlife and biodiversity, providing habitat for 2300 different species from birds to bryophytes, invertebrates and lichens, fungi and mammals.

Despite all they provide for us our oak population is under threat and needs our urgent protection. Pests and diseases. Climate change. These threats carry the potential to do serious harm and even devastate the oak population that has always simply been there for us. It is, therefore, imperative that we can deepen our understanding of these threats to be able to take proactive action to protect our oaks now and for years to come.

Action Oak's mission is "to lead the vital work and research to project our native oak trees and safeguard their future" and this work could not be more important. Supporting and connecting research communities in their work to explore the intricate problems facing our oak trees; Acute Oak Decline, Oak Processionary Moth, powdery mildews, heart rot fungus and more, exploring the effects of climate change induced storm events on our oaks and understanding what drives oak masting to mention just some of the vital research that Action Oak is involved with.

Trying to picture our landscape without oaks in it is a sobering thought and a reality that we cannot allow to happen. This 2023/24 Annual Report shows not only the passion and knowledge of those who are involved with Action Oak but also demonstrates the categoric importance of being able to reach the source of the threats and understand how to mitigate them.

Please read this annual report, learn more about their work and lend your support to this important cause.

1 Forestry Commission. (2018) Preliminary findings of the extent, composition, health and nature of woodland oak in Britain.

Introduction

Geraint Richards, Chair, Action Oak

It is exciting to see the third Action Oak Annual Report published, highlighting the important work that has been carried out over the past year by a range of experts with a vitally important common goal, to protect our native oak trees that are threatened by an alarming array of pests and diseases. I want to thank all of those who continue to devote their incredible talent to this important cause.

A significant milestone for Action Oak during the past year was the appointment of Annabel Narayanan as our Project Director, thanks to generous funding from Defra. We welcome Annabel to the team where she joins Sarah and Georgina, all working together at the hub of the Action Oak wheel!

Last summer we had an excellent Action Oak Partner Event at Birmingham University and in the autumn some of the Committees' members were hosted at Norbury Park; my thanks to both organisations for kindly providing such excellent venues for meetings and site visits.

As we look to the coming year, the scale of the challenge - 'Protecting our Oak Trees' - is huge indeed and hence why your continued support of Action Oak is so important and, I can assure you, so gratefully received. I want to take this opportunity to thank all of you and I look forward to meeting many of you over the next twelve months.

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

Kate Halstead, Newcastle University

Figure 1: A 3D point cloud of storm damaged oak (crown damaged) acquired using a GeoSLAM ZEB Horizon LiDAR scanner.

In recent years (~20 years), European forests have experienced an increase in disturbance events, as a result of both climate change, and changes to forest composition and structure post World War II (Senf and Seidl, 2020). Of such disturbance events, storm damage has been identified as one of the most important abiotic factors, with the ability to cause stand scale canopy removal and immediate tree mortality (Pawlik & Harrison, 2022). A census of storm damage to native UK oak (*Quercus petraea* and *Quercus robur*) was conducted across three sites in the North East of England, in the aftermath of two severe successive winter storms, named *Arwen* and *Barra*. The region was one of the worst affected in the country; it suffered considerable losses to veteran and notable oak specimens and recorded some of the highest windspeeds in both storms. This is of concern given the species ecological and cultural significance (Quine *et al.*, 2019).

A key aim of our research was to characterise the impact of the two successive storms, *Arwen* and *Barra*, across three local study sites, and examine different scales of storm damage, crown, windsnap and windthrow (Fig. 2). The study was carried out across three sites: The Wallington Estate, Northumberland, Cockle Park Farm, Northumberland, and Gosforth Nature Reserve (GNR), Tyne and Wear. Oak was distributed in differing settings at each site i.e., woodland, parkland and hedgerow.

A ground-based mobile LiDAR (Light detection and ranging) scanner, a GeoSLAM ZEB Horizon, was utilised to scan individual damaged oak trees, post-storm, and create a 3D structural point cloud (Fig. 1).

Several parameters were extracted from the point clouds, including: orientation in relation to the direction of the storm attributed to damage, DBH, height, crown area, crown volume, and, the root plate depth and diameter of windthrown oak (Terryn *et al.*, 2022). In addition to individual tree scans, damaged oak with above ground failure (i.e., windsnap and crown damage) were also examined for presence of points of pre-existing structural weakness. This included bark defects (bark stripping, loose bark or cankers), weak forks and old pruning wounds.

In the UK, the majority of gale and storm force winds track from a south-westerly direction which corresponds with the UK's prevailing wind direction. Analysis of hourly wind data, extracted from MIDAS (UK Met Office Integrated Data Archive System), at Redesdale Camp weather station, Northumberland, indicated that both storms, *Arwen* and *Barra*, came from a non-prevailing direction. Max gust speeds of *Arwen* were recorded at >32m/s, and the tracked from the North, while max gusts of 15m/s were recorded for *Barra*, which tracked from a south-easterly direction. Hence, of the two storms attributed to oak damage, *Arwen* was significantly more severe than *Barra* in terms of recorded average wind and maximum gust speeds. When compared with the wind speeds of other Northumberland storm events between 2011-2021, it is evident that *Arwen* was the most powerful storm to have taken place within this time frame.

The majority of debris of the damaged oak was oriented in an Easterly to Southerly direction (Fig. 3). This suggests that a storm tracking from a Northerly direction, i.e., *Arwen*, was responsible for the damage. Across all sites, *Arwen* was attributed to causing 92.4% of all damage incidences, indicating a clear dose-response relationship between storm severity and the scale of damage. A total of 79 storm damaged oak were recorded across all three ~80ha study sites, with crown damage being the most recorded damage type at 59.5%, followed by windsnap, 22.8% and windthrow, 17.7%. Bark defects were the most recorded structural weakness point, making up 48.3% of all reported defects, followed by weak forks, 40% and prune wounds, 11.7%.

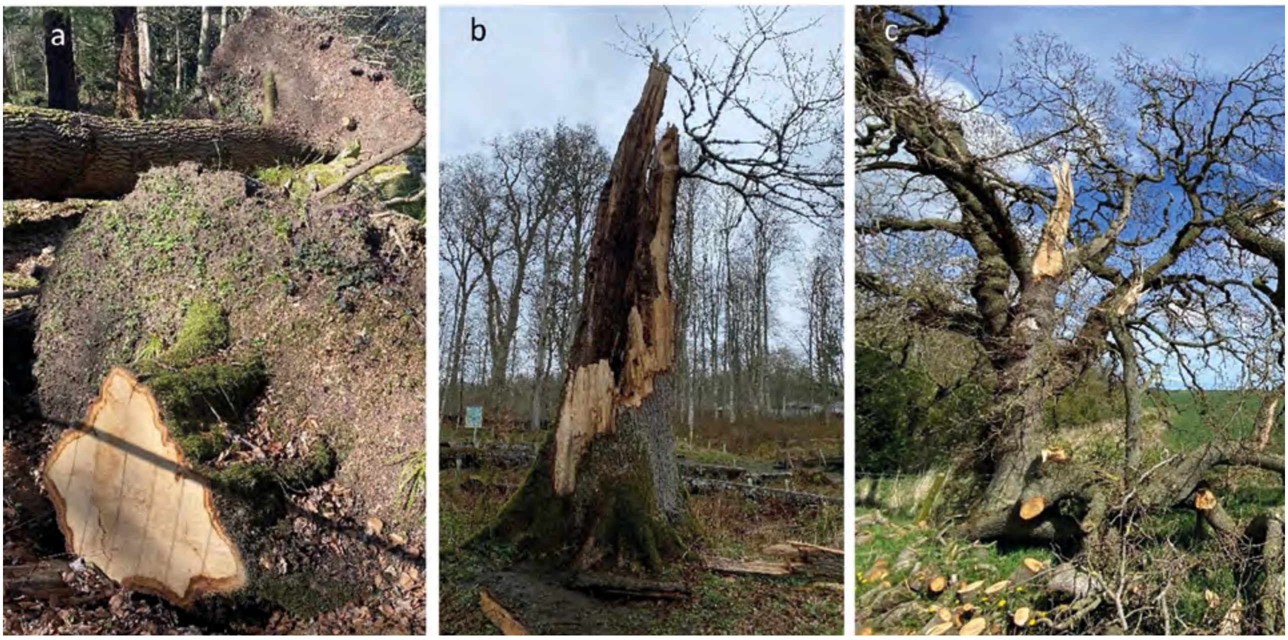

Figure 2: Differing scales of storm damage to oak: a) windthrow, b) windsnap, and c) crown damage.

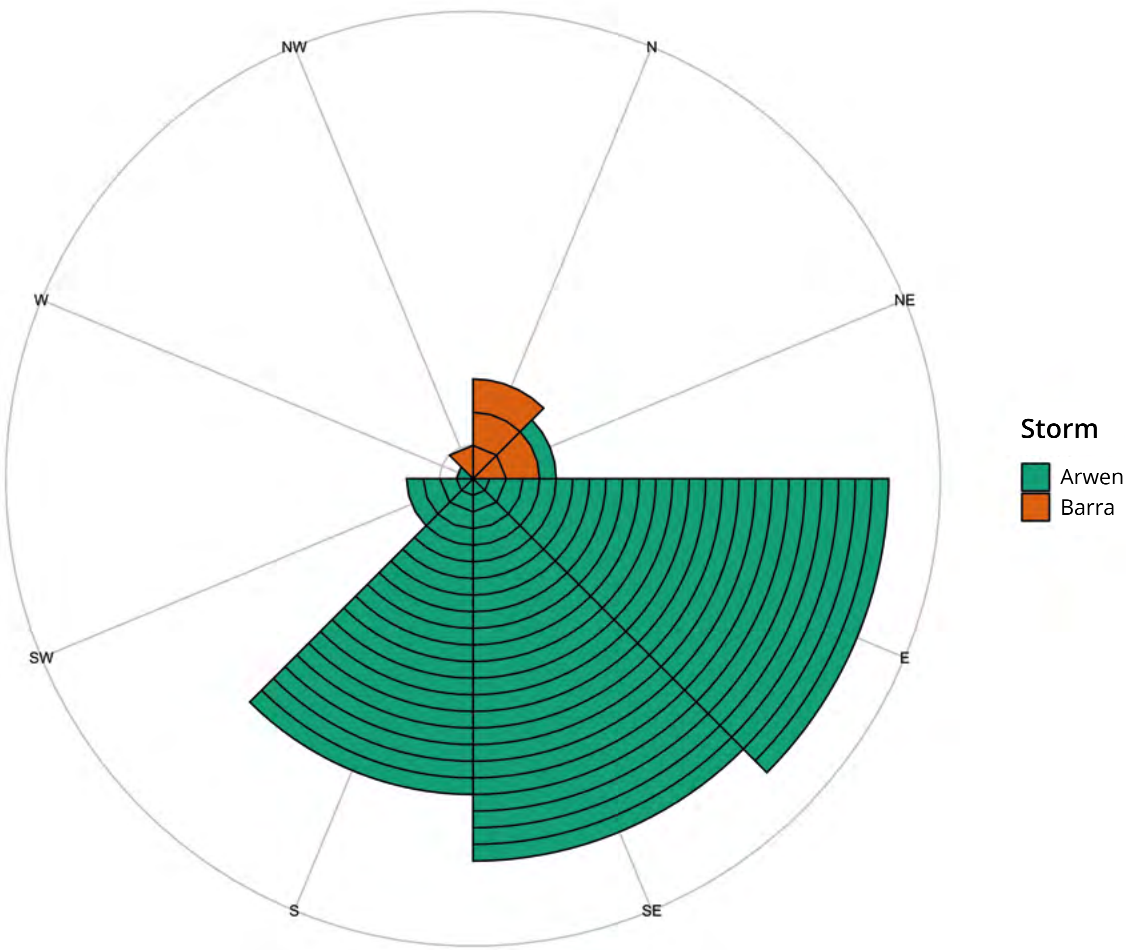

Figure 3: Wind rose diagram to show the direction of branch/tree fall and the corresponding storm attributed to damage.

There were several marked differences between our findings and those documented following the *Great Storm of 1987*, the most comparable storm to *Arwen* in terms of severity and scale of damage to trees (Cutler *et al.*, 1990; Gibbs and Greig, 1990). Prune wounds were recorded in low abundance, suggesting that past site management was not a factor influencing the incidence of damage. In addition, there was a high recorded incidence of wind snapped native oak in comparison to the *Great Storm of 1987*. Weak forks may have contributed to the significant number of wind snapped oak recorded in the survey, as 39% of all documented weak forks failed at the forked weakness point, resulting in windsnap. The high incidence of windsnap, attributed to Storm *Arwen*, also fits our informal observations of storm damage in other parts of Northumberland. As discussed, the rare non-prevailing wind direction of *Arwen*, is likely to have influenced such differing observations to comparable storm events, such as the *Great Storm of 1987*.

Our findings stress the importance of documenting rare observations of storm damage, from a non-prevailing wind direction, to oak at a local scale, to facilitate improved modelling of future storm events and inform forest management. The next stage of the research will entail the completion of a statistical wind risk model to identify site specific predictor variables, those factors most likely to predispose native oak to future storm damage.

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.

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From Metabolomics to Bacteriophages: Exploring strategies for managing Acute Oak Decline

Emily Grace, Vanja Milenkovic, Diana Vinchira-Villarraga, Robert W. Jackson[1], Mojgan Rabiey

UNIVERSITY OF BIRMINGHAM | BIFOR BIRMINGHAM INSTITUTE OF FOREST RESEARCH

1 *School of Biosciences and the Birmingham Institute of Forest Research, University of Birmingham, Birmingham B15 2TT, UK*

Acute oak decline is a severe disease that affects UK native oak species (*Quercus robur* and *Quercus petraea*), impairing their growth, productivity, and lifespan. 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 AOD, our research group has established different 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 and field 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.

To identify how oak trees respond to AOD pathogen infection, we intended to follow

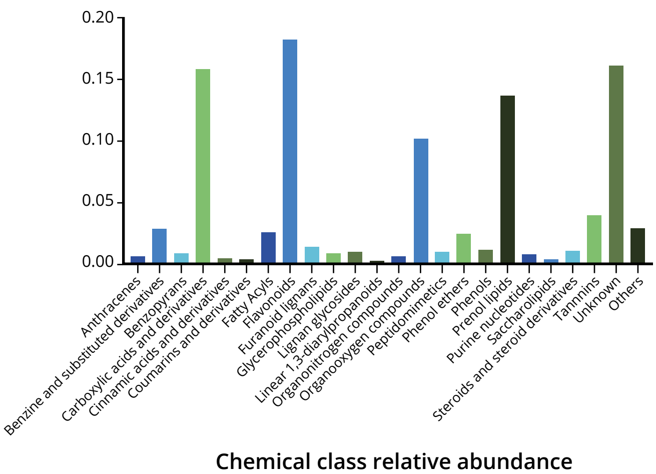

Figure 1: PhD student, Emily Grace, sampling oak woody tissue (right) for metabolomics analysis (left) and phages isolation

the changes in the metabolism of healthy (asymptomatic) and diseased (symptomatic, observed as having oak bleeding canker) trees using untargeted metabolomics. This project started with the analysis of the metabolic profile of healthy trees of ages ranging from 3 to 80 years old sampled in Norbury Park Estate (Figure 1). Through this analysis, we characterised the major chemical families (and metabolites) of oak wood (Figure 1). Once identified, we tested if these healthy wood extracts had any antibacterial activity against Bg, Gq and Rv. The results showed that the healthy wood extracts reduce the growth of the AOD bacteria, as well as the growth of different *Pseudomonas syringae* and *Pseudomonas savastanoi* pathovars (all of them pathogens of trees), indicating that healthy trees synthesise metabolites with antibacterial activity. A correlation analysis allowed us to propose that the metabolites responsible for such activity belonged to the tannins, prenol lipids, and terpenoids chemical classes, and current efforts are being made to prove the identity of these compounds and determine if they can be used on trees to reduce the severity of AOD or if treatments of trees can induce their production.

With this information, we moved on to study the metabolic profile of healthy and symptomatic trees, as well as their antibacterial activity, focusing on the candidate antimicrobial metabolites selected in the first phase. Remarkably, the extracts from symptomatic tissue of diseased trees (Figure 2) showed a higher antibacterial activity than those observed in healthy tissue from diseased trees and woody tissue from asymptomatic trees (Figure 2). Alongside the changes in the activity, a higher abundance of certain ellagitannins was also observed in the symptomatic tissue, suggesting a role of this type of metabolites in the response of oak during AOD development, at least at a local level. Further analysis is being carried out to identify other metabolites that are up or down-regulated (i.e. are more or less abundant) in healthy and symptomatic trees to determine which other metabolites are being affected during the disease progress and whether there

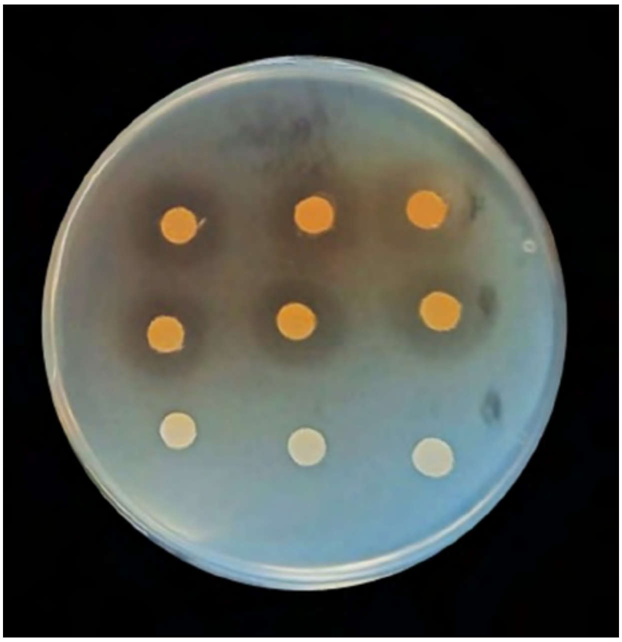

Figure 2: Oak bleeding canker from a specimen infected with AOD (right) and its antibacterial activity against Brenneria goodwinii (left)

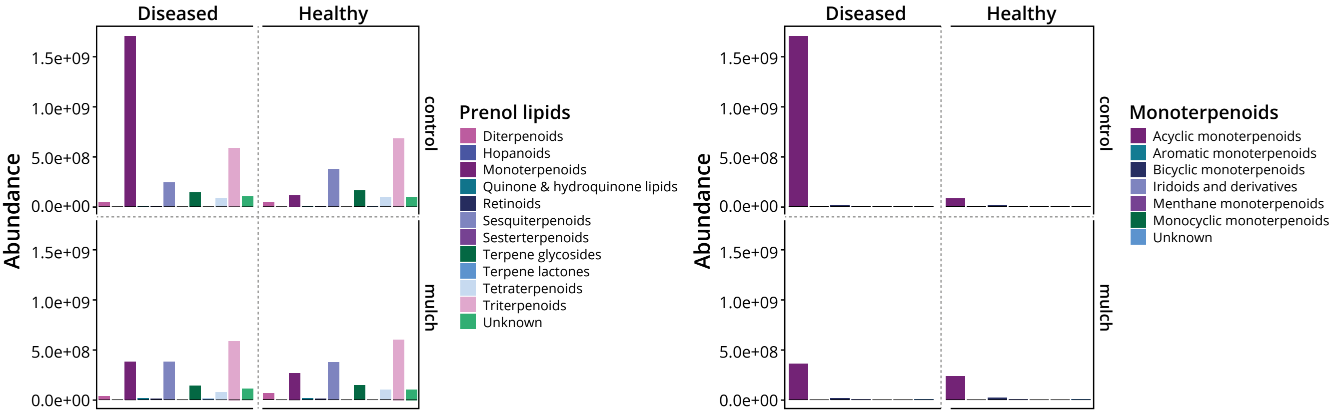

Figure 3: Abundance of prenol lipids and monoterpenoids in oak leaves

are or not, systemic responses to AOD in the symptomatic trees.

On the other hand, linked to the second and third aims, we developed a field project to identify the triggers of tree health decline and the mechanisms underlying tree resistance to, and recovery from, disease. Conducting a three-year field experiment, we monitored 24 oak trees across two woodlands. Half of these trees exhibit clear symptoms of AOD, while the other half remain asymptomatic. At each site, half of the symptomatic and asymptomatic trees have undergone mulching treatment.

Since 2022, we conducted biannual visits to the sites to collect phenotypic measurements of the trees, as well as soil and leaf samples for microbiome and metabolome analyses. The microbiome analysis has revealed significant differences between diseased and healthy trees. Both bacterial and fungal community compositions in the soil and leaf samples collected from Wyre Forest's oaks exhibit distinctions between healthy and diseased individuals. To date, no discernible effect of the treatment has been observed. Monitoring of the trees will continue for another year to assess the outcome of the applied treatment.

Metabolite data indicates an upregulation of prenol lipids (isoprenoids), particularly a heightened accumulation of monoterpenoids observed in diseased oaks, specifically within the diseased control group (Figure 3). These compounds play a pivotal role in the plant's stress response to high temperatures and drought and in defence against herbivores and pathogens. Mulching symptomatic oaks has significantly reduced the production of acyclic monoterpenoids, including the compound pterogynidine. This decrease in monoterpenoid levels may indicate either a potential improvement in tree health (suggesting a reduced need to combat infection) or a consequence that could potentially impact tree health negatively—further tree health surveys will help clarify this. Overall, mulch treatment shows promise in mitigating drought and heat stress in oak trees and, consequently, in addressing Acute Oak Decline. Continued monitoring and analysis will provide further insights into the treatment's effectiveness.

The fourth aim of the research group is to determine how bacteriophages, viruses that infect and kill bacteria, impact AOD-related bacteria species and if they can be used to prevent and control oak bacterial canker in infected trees. Thus far, phages infecting *Bg* and *Gq*, named BREN and GIB phages, have been isolated from diseased lesion tissue of oak trees from the Midlands. These phages have been characterised to determine their key biological factors, such as their virion morphology via transmission electron microscopy, growth

© Action Oak

pattern via one-step growth curves, genetic features via genome sequencing, and plaque (zones of lysis) morphology. Furthermore, they have then been investigated for their usability as biocontrol to reduce disease in trees. Our study has found that both BREN and GIB phages can successfully prevent the growth of *Bg* and *Gq*, respectively, under lab conditions. Both phage types solely infect their host species, so they would not harm the wider microbiome as other antimicrobials do and can lyse several strains of their respective hosts. As the combined presence of both *Bg* and *Gq* causes oak bleeding cankers, an ongoing study focuses on how the phages impact *Bg* and *Gq* when they are placed together within a community. Glasshouse trials using oak saplings will also be performed in Spring 2024 to assess how effective the phages are in preventing the growth of each bacteria alone or in a community *in planta*.

Can we predict when bumper acorn crops will occur?

Dr. Andrew Hacket-Pain, University of Liverpool

We are rapidly making progress in our ability to forecast bumper seed crops years in trees ("mast years"), but as is so often the case, oaks are proving a tricky nut to crack.

Acorn crops are highly variable from year-to-year, a characteristic known as masting, which oaks share with many other temperate trees. The reasons for this "boom-and-bust" reproductive strategy are still to be fully unravelled, but we know that cycling between years of starvation and satiation helps to reduce losses to specialist seed predators. In oaks these include acorn weevils, whose characteristic drill holes can often be spotted in mature acorns (break such an acorn open and you might spot the resulting weevil grub). The years with small acorn crops starve populations of these seed predators, so that in mast years the remaining population is overwhelmed, and a higher proportion of seeds escape unscathed. In mast years, seed consumers like the jay cache so many acorns that a larger number are forgotten, increasing the number of "planted" acorns and enhancing seed dispersal. We also know that concentrating flowering effort in occasional bumper years increases pollination success, so more of the female oak flowers produced in spring go on to develop into mature acorns.

This variable acorn production has many wider consequences, including cascading effects through food webs. For example, woodland mammals and birds have higher winter survival rates after mast years, and are in better condition for breeding the following spring. Masting is also a challenge for the tree nursery industry, as it creates an unpredictable annual supply of acorns for planting. In oak, the technical challenge of long-term acorn storage exacerbates this challenge – shortages in acorn supply cannot be met from stored reserves of seed, as can be done for conifers with high seed storage potential. Recognising this problem, and its potential impact on national woodland planting ambitions, Defra recently funded a Treescapes Fellowship (part of the "Future of UK Treescapes" research programme), to work on evaluating possible solutions. In partnership with Action Oak, I have been exploring the potential to develop forecasting tools that we could use to predict bumper and failure crops. The successful development of such tools, enabling a forecast of autumn acorns crops months in advance, would enable improved planning in the industry. For example, seed collectors would know which areas of the country to target for their annual seed collection campaigns, and tree nurseries could manage orders and stock, knowing the likely acorn supply next autumn.

In the last few years, we have made great strides in the forecasting of masting, working initially with beech, and more recently – as part of the Fellowship – with a range of important broadleaf and conifer species in collaboration with colleagues in Vienna, Austria. However, oak is proving more challenging than other species. Unlike most other species, acorn crops in oaks seem to be determined by a suite of different process, whose individual importance varies

Image © Nick Fewings, Unsplash

according to local growing conditions. A recent study from France showed that annual acorn production in forests growing under oceanic climates depended largely on the number of flowers produced in the spring – large numbers of flowers translated predictably into larger numbers of mature acorns. This is a similar process to the regulator of masting in beech. It suggests that advanced forecasting of acorn crops might be achievable in oaks too, if we can understand what climatic factors determine their annual flowering effort. However, the same study showed that for trees growing in continental, things were different, and the number of flowers produced in spring was unimportant in determining the acorn crop. Instead, in these populations the weather conditions in April were key, by determining the pollination success and therefore the proportion of flowers that developed into mature acorns. Adding to this complexity, we are currently finalising a new analysis that demonstrates the central role of late spring frosts on regulating acorn production in oak forests growing in the French Pyrenees (late spring frosts – perhaps counterintuitively – may become increasingly important under climate change). Ongoing work that started as part of this Fellowship will attempt to pin down the complexity of oak masting, by focusing on weather conditions during the flowering period – which varies year-to-year. Using this approach, we hope to identify in which regions the successful forecasting of oak masting will be possible.

One of the challenges with understanding (and predicting) acorn production in British oaks is the lack of a systematic and detailed acorn monitoring programme in the UK. This is in contrast to France and elsewhere in Europe, where oak flowering and acorn production are monitored across large networks of sites. In

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the UK this gap is partly filled by The Woodland Trust through Nature's Calendar, a citizen science programme that focuses on phenology but includes data on autumn seed and fruit crops submitted by members of the public. Also, detailed monitoring of acorn crops in the UK has expanded in the last few years – not least thanks to the efforts of the Action Oak-funded PhD student Ryan McClory, who has been monitoring oak at Wytham Woods. Nevertheless, a wider and more detailed monitoring programme would provide us with vastly improved data on oak masting patterns across the UK. As well as helping us understand this intriguing natural phenomenon, such monitoring would enable us to identify the impacts of climate change and other threats (such as oak lace bug) on acorn production in our oak forests. Each oak in Britain originates as an acorn, so understanding how they are produced must be a priority if we are to safeguard the continued presence of oak in our landscapes.

More information about the project can be found here: **https://www.uktreescapes.org/projects/dr-andrew-hacket-pain/**

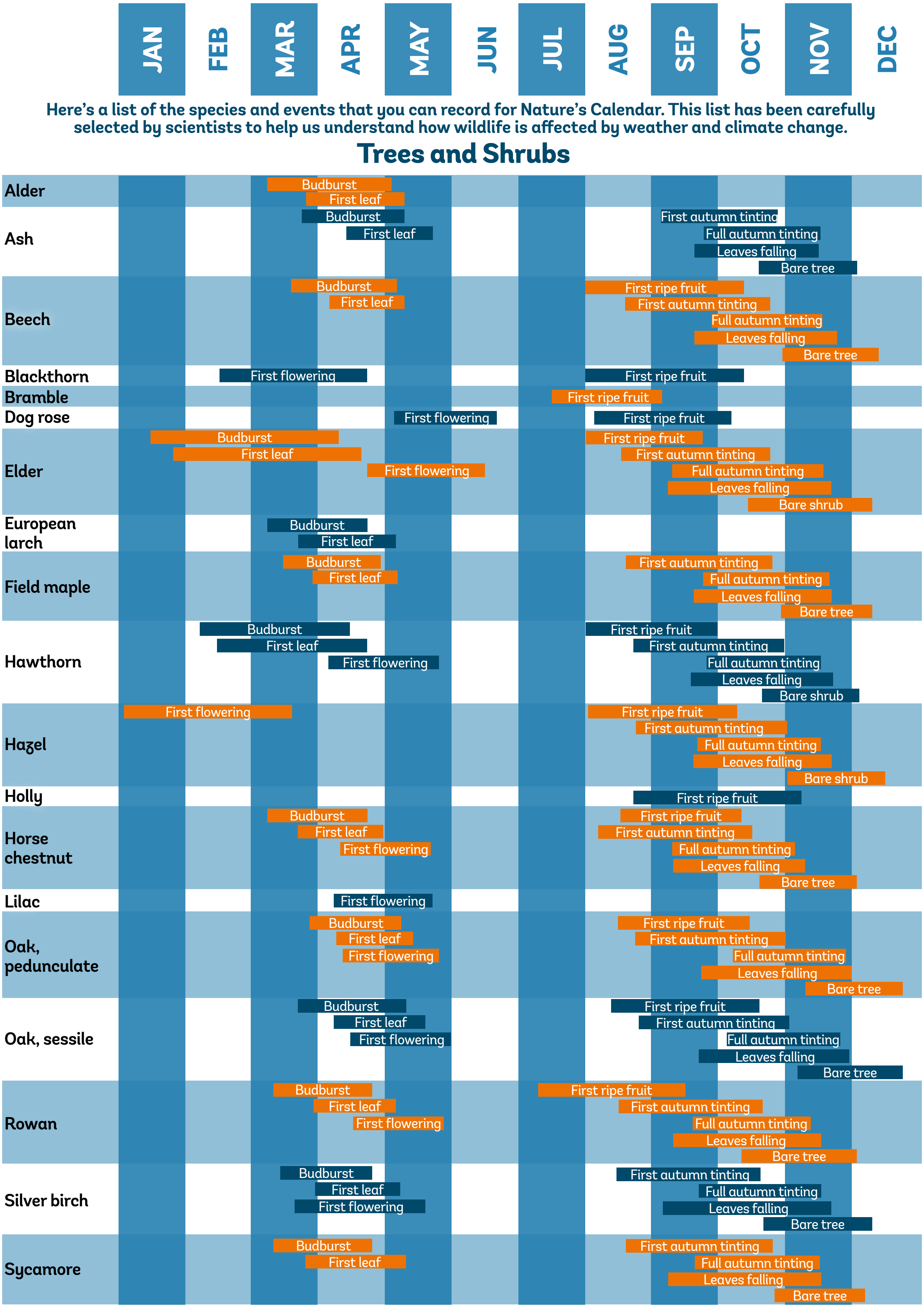
Opposite: part of the Woodland Trust's Nature's Calendar which collects information from the public on certain species to monitor the effects of climate on nature

Nature's Calendar

naturescalendar.woodlandtrust.org.uk

Here's a list of the species and events that you can record for Nature's Calendar. This list has been carefully selected by scientists to help us understand how wildlife is affected by weather and climate change.

Trees and Shrubs



Genetic characterisation of oak (Quercus spp.) for breeding and conservation

Eamonn Cooper, Trinity College, Dublin
PhD research project

INTRODUCTION

Oaks *Quercus robur* and *Quercus petraea* are a highly valuable organisms from both ecological and economic perspectives across the UK and Ireland. Their utilisation in the lumber industry, as well as their afforestation usage, has been underappreciated in the previous decades but renewed appreciation of their value towards environmental stability, the woodland economy and biodiversity has been growing in recent years. Management of oaks in Ireland and the UK has varied throughout history resulting in our current population being fragmented in varying sized forests throughout the region (Ward 2021, Department of Agriculture 2022).

Preservation of high-quality breeding stocks began initially in the early 1980s where successive masting years resulted in high amounts of saplings being preserved in living archives (Felton *et al.* 2006). In the subsequent decades, assessment of native stock for plus trees began the process of tree improvement. This has culminated in the assemblage of just under 250 plus trees from across the UK and Ireland in multiple clone archives in addition to several progeny trials of their offspring by Future Trees Trust (FTT) and the Forest Genetic Resources Trust (FGRT) (Hubert and Savill 1999, Felton and Thompson 2008, Quine *et al.* 2019). It is here where this study begins. The genetic makeup of this cohort is yet unknown. **This research therefore aims to assess the genetic diversity of the current breeding stock**

selected by FTT and FGRT. As *Q. robur* and *Q. petraea* are difficult to identify with certainty and will readily hybridise with each other, the initial step in genetic analysis will be to correctly assign species and to quantify hybridisation and introgression in this cohort. Additionally, as the long-term goal of tree improvement is to develop genetically improved varieties for forestation, the preservation of genetic diversity is needed to prevent population bottlenecks. It is imperative to know if the selected cohort is representative of current genetic diversity across the region. This will be accomplished through Single

Nucleotide Polymorphism (SNP) sequencing using a combination of recently created SNP reference datasets and GenBank sequences as a comparative population.

The heritability of the attributes that were used to select plus trees has yet to be assessed. **This study will aim to examine our ability to measure such traits and define these within progeny trees whilst assessing their heritability.** This will involve investigation of morphological traits of progeny trees with comparison to similarly planted provenance trials across multiple locations.

Additionally, in recent years the impact of introgression and its potential relevance to native populations for adaptation has begun to be better understood (Degen *et al.* 2023). The directional flow of genes from *Q. robur* to *Q. petraea* in addition to others within Europe since the post glacial recolonisation is yet to be fully understood. There is potential that syngameon network exist between species acting as complex mechanism of adaptation over large periods of time with individual species acting as genetic reservoirs for adaptation (Cannon and Petit 2020). The consideration of this in addition to many other factors must be a crucial aspect of tree improvement within oaks. In the plus tree collection, these genomic regions may provide future adaptive ability, understanding this may be the factor that ensures the future success of oak improvement. It's my intention to add to our understanding of these complex issues through the incorporation of genomic and morphological assessment.

PROGRESS TO DATE

Overall progress has been good despite some setbacks. I am well on my way to my goal to be able to make recommendations surrounding species identity and degree of introgression with the work in the last six months coming together well. Progeny trial demonstration line samples were sent out for sequencing and the raw data received in late-January 2024. In addition, recent publications in the field from November of 2023 have necessitated repeating and altering quality control parameters in the bioinformatic data analysis. Once the additions to the UK based reference data are collected and sequenced in the coming growing season we will be able to conclude the research in this section of the project. Definite conclusions can then be made and recommendations based on the genetic characteristics can be made.

FUTURE WORK AND QUESTIONS THAT NEED TO BE ANSWERED

There are many avenues of research and questions that this project has yet to investigate. This report contains data that has only recently been generated and a full understanding is yet to be achieved.

Going forward I would hope to answer the following questions:

- Is our SNP panel sufficiently complex to distinguish parental haplotypes in the progeny trial trees
- Can we measure the biases in sNMF species assignment and remove the influence of geographic differentiation in the species assignment?
- How do the non-native Quercus species interact with the Plus trees? Will they be relevant to our cohorts?
- Is the large observed difference in the plastid haplotype groupings a result of a previously un-observed batch effect or a true reflection of the data?
- Is the separation of species in seed orchards necessary from a scientific perspective given what we know about plasticity of the genome?
- How does the genetic characterisation correlate with phenotypic features?

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News on AOD Research

Sandra Denman, Forest Research et al.
March 2024

Figure 1: The adult female oak jewel beetle Agrilus biguttatus

Last year I wrote to readers explaining that we had been the lucky recipients of a research grant in 2020. This year I am writing to tell you that the research project is over now, and I will share some of the results with you here. Before doing so I would once again like to thank the organisations that financed the project, which were Defra, BBSRC, NERC and the Scottish Government, and we would also like to thank Action Oak for its support.

The key aim of the research was to determine whether the native oak jewel beetle (*Agrilus biguttatus*) (Figure 1) is essential to the development of Acute Oak Decline (AOD) or not. AOD is a complex disease characterised by black weeping stem patches with the underlying tissue macerated and the larval galleries of *A. biguttatus* always present in association with the rotting lesions. The stem degradation is caused by a bacterial complex (a pathobiome) comprising at least three species of bacteria, but primarily *Brenneria goodwinii* is the species shown to have a very damaging effect due to a large number of pathogenicity genes, and it is supported by *Gibbsiella quercinecans* that seems to produce cell wall degrading enzymes and *Rahnella victoriana* whose role is not yet certain.

The larvae of *A. biguttatus* obtain entry to live inner bark tissues of oak when adult females lay eggs on the outer bark of mature oaks. The beetles are thought to colonise trees in a weakened state and it is considered that they cannot ingress healthy oak trees. The general view about the natural behaviour of *A. biguttatus* prior to the advent of AOD considered it responsible for hastening the death of weakened trees by the girdling action of larval feeding on live inner bark tissues. *A. biguttatus* has a cryptic life cycle where larvae feed and develop in the innerbark over 2 years, and by chewing through the inner bark they form tunnel-like galleries until they are ready to pupate. After pupation, adult beetles leave the trees through D-shaped emergence (exit) holes. In this natural pre-AOD condition, no weeping stem patches are formed on oak stems. AOD was first seen in the UK around the late 1970s and early 1980s. Since then, overall, it's incidence and distribution have been increasing. Many thousands of oak trees are affected and most die although some do survive. The role of *A. biguttatus* in AOD has been controversial and we wanted to find out if AOD can occur in the absence of *A. biguttatus*.

In the laboratory logs were inoculated with the eggs of *A. biguttatus* and after feeding for 8 weeks, larvae were dispatched and washed in methanol to extract chemicals produced on their body and mouth parts. The methanol was dried off leaving pure chemical fractions, which were tested to find out whether they influence the activity of the bacterium, *B. goodwinii*. The various fractions were added to different test-tubes containing the bacterium in minimal growth medium and the growth of the bacterium over 48 hours was measured, and the active functional bacterial genes were examined through transcriptomic analyses. We demonstrated that chemical cues from *Agrilus* larvae caused an increased growth rate and final cell density, as well as the differential expression of almost 800 genes in *B. goodwinii*, including come pathogenicity genes (Figures 2A and 2B).

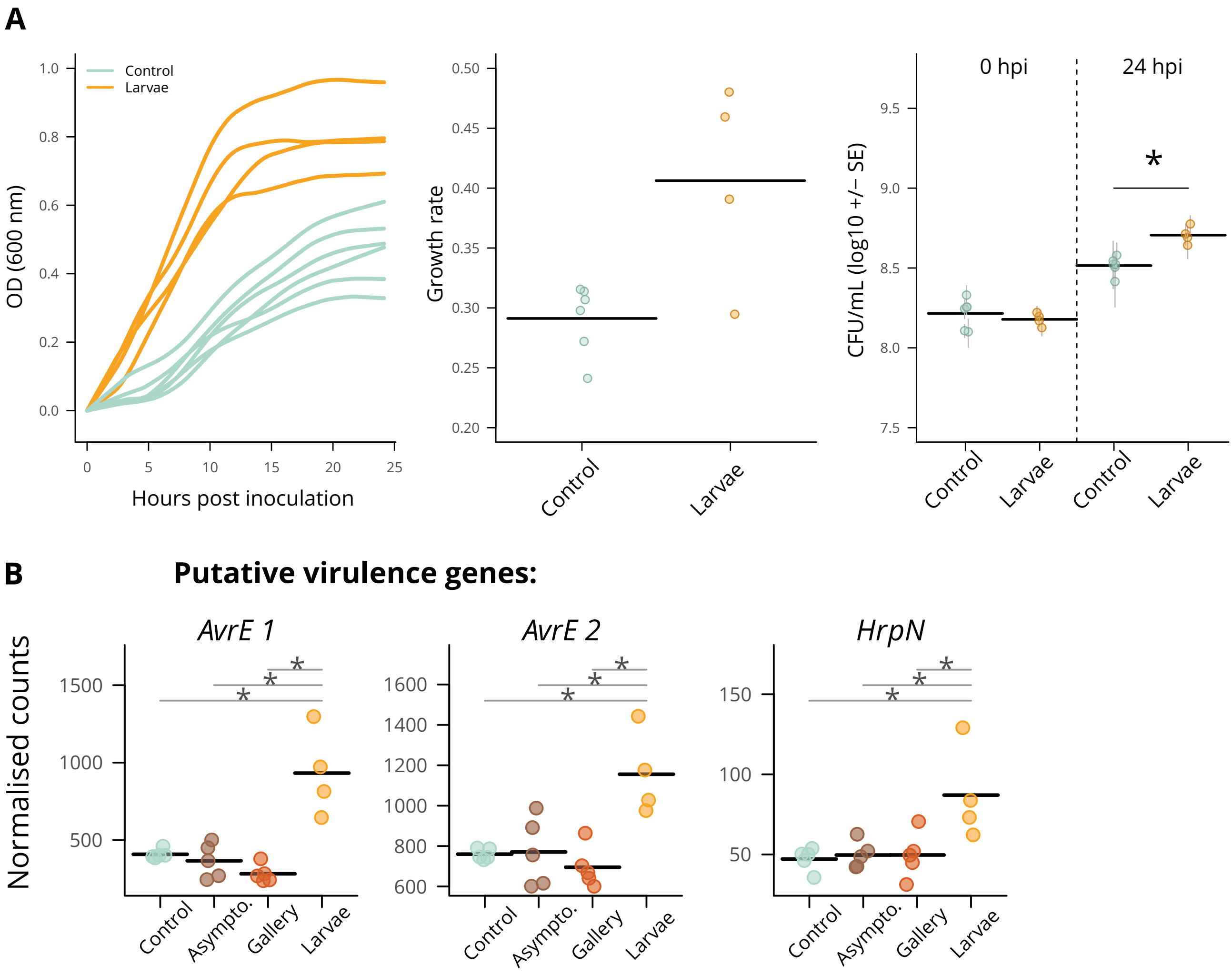

Figure 2: (A) Results of bacterial growth tests in minimal medium amended with larval extract (yellow lines/ dots) or not (Control blue lines/dots) and (B) Gene expression in tests

This indicates a direct link between the larvae and the bacterium where the interaction causes a change in the behaviour of the bacterium potentially causing it to become pathogenic. At this stage no other causes of upregulation of these pathogenicity genes in *B. goodwinii* are known. Thus, this study suggests that *A. biguttatus* larvae are essential to AOD and indeed an integral part of the disease. This study was carried out in partnership with Rothamsted Research chemical ecologists Gareth Thomas, John Caulfield and Jozsef Vuts, and Birmingham University microbiologists Marine Cambon and James McDonald. Grateful thanks are also given to the technical support we receive from FR TSU groups based at Alice Holt and Fineshade.

Assessing pathogenicity of bacteria isolated from stem bleeds on broadleaved trees

Bridget Crampton

The bacterium, *Brenneria goodwinii*, plays an important role in lesion development in trees affected with Acute Oak Decline (AOD). Work package 4 of the BAC-STOP project aimed to determine if *Brenneria* species as well as other AOD associated bacteria (*Lonsdalea britannica*, *Rahnella victoriana* and *Gibbsiella quercinecans*) were involved in lesion formation and subsequent stem bleeds in non-oak broadleaved trees. To this end, we screened 158 swabs taken from stem bleeds on 24 different trees species with a multiplex real-time PCR assay that identifies AOD-associated bacteria. Fifty swabs tested positive for at least one of the AOD-associated bacteria. In addition, we were able to isolate AOD-associated bacteria from swabs and diseased stem material including *B. goodwinii* from lime (*Tilia* sp.), birch and horse chestnut; *R. victoriana* from lime, birch and beech; and *G. quercinecans* from lime stem bleeds.

In order to determine whether these isolated AOD-associated bacteria are involved in lesion formation in non-oak trees, we inoculated small-leaved lime (*Tilia cordata*) and oak saplings with *B. goodwinii* strains isolated from lime and oak and noted that *B. goodwinii* formed lesions on oak saplings but not on lime saplings. This was further confirmed with log inoculation trials where oak, lime, beech, birch and hornbeam log billets were inoculated with *B. goodwinii*, and at separate inoculation points, a mix of AOD-associated bacteria (Figure 3). Statistical analysis of the lesion lengths on inoculated logs showed that *B. goodwinii* and AOD-associated bacteria play an important role in lesion formation in oak but less so in other tree species.

Hornbeam Oak Lime Beech Birch

Figure 3: Broadleaved tree log billet inoculations with AOD associated bacteria (above) and subsequent lesion formation (below)

As part of this work package, we have also written and produced fact sheets on tree diseases caused by *Brenneria* and *Lonsdalea* species (New Bacterial Tree Disease Factsheets – Bacterial Plant Diseases Programme). These fact sheets are useful for identification of tree diseases in the field. Furthermore, we have written four peer-reviewed papers that taxonomically describe new bacterial species isolated from broad-leaved tree stem bleeds.

Do volatile compounds attract the bacteria associated with AOD?

Jozsef Vuts

As well as demonstrating that *A. biguttatus* larval chemical elicitors make *B. goodwinii* bacteria grow faster and become more pathogenic, we have established that female adult *A. biguttatus* prefer the smell of AOD tree foliage over healthy tree foliage, and that gravid females find the volatiles produced by AOD bacteria attractive. These discoveries indicate that the tree, bacteria and beetle are ecologically tightly linked, and such interactions are governed by naturally occurring chemicals. We have collected volatiles from symptomatic and healthy tree foliage across England and separated the component parts of the volatile bouquets with gas chromatography (GC). GC-mass spectrometry (Figure 4A) then identified the constituents, and a synthetic blend of AOD tree-specific volatiles made healthy oak leaves more attractive to beetles than oak leaves alone in lab olfactometer behavioural assays. Furthermore, we have shown that the volatiles collected from the headspace of AOD bacteria cultures are also attractive. We even used the beetle's antenna to indicate which specific components of attractive bacterial headspace elicit responses and have located several volatiles within the mixture which elicit antennal responses and thus could be attracting the beetle. As some of these are novel volatiles, we synthesized them ready for new behavioural experiments to demonstrate the attractiveness of the synthetic compounds through olfactometry assays (Figure 4B) and field tests. It was important to show that the produced bacterial volatiles are not only emitted by pure cultures on agar plates but also from AOD lesions, which we have achieved, highlighting that the identified bacterial volatiles do play an ecological role in the natural environment of *A. biguttatus*. This knowledge could enable the development of improved monitoring tools for the beetle through optimising semiochemicals-based lures.

Monitoring AOD

Nathan Brown

2023 was a year with very high numbers of oak trees with AOD lesions. At our seven long term

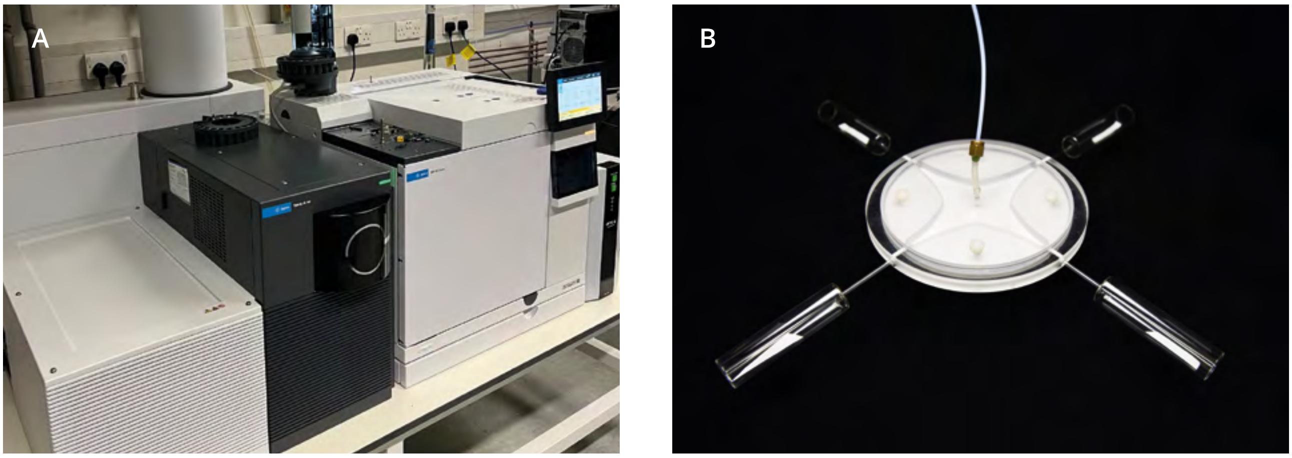

Figure 4: (A) Coupled gas chromatography-mass spectrometry (GC-MS) (B) Four-arm olfactometer to measure A. biguttatus behavioural responses to volatiles.

monitoring plots we have recorded increasing numbers of symptomatic trees each year since 2018. At three of the plots, we recorded the highest numbers of affected trees since monitoring began in 2009. The increase in stem bleeding was mirrored by an increase in new D-shaped exit holes, highlighting a correlative trend between the two main symptoms and signs of AOD.

At the national scale we continue to receive new reports of AOD affected trees through tree alert. Each new record helps to improve our understanding of the distribution of AOD and site factors that make oak trees more likely to be predisposed to the bacterial and insect species associated with AOD. In 2018 we produced a risk map for AOD across England and Wales using a designed survey and 207 Tree Alert reports of AOD. Over the last year we have worked to update this prediction to cover all Great Britain, using additional observations from the oak condition survey and a further 149 records from Tree Alert. We aim to publish this work next year, in addition to the extended geographical coverage the new analysis should more accurately describe trends with environmental variables due to the increased number of observations supporting the model.

This summer we plan to run training courses to aid the detection of affected trees among volunteers, forestry professionals and members of the public so that we can better understand how easily symptoms can be recorded and further improve our risk maps.

Working with stakeholders

Liz O'Brien

We held an important all-day workshop in London with stakeholders in November 2023. The aims of the event were to work with key stakeholders to start discussions on co-developing a decision tree / framework, to support consideration of management decisions and potential actions for oaks in different contexts: such as oaks in parkland, in mixed woodland and in urban areas. We also wanted to think through and discuss key elements of land manager decisions that could feed into a decision

Figure 5: (A) Key issues and actions to address those issues as discussed by stakeholders where problems were listed on pink cards and responses to solve the problems matched on green cards (B) Stakeholders at the workshop

tree and potential actions. Over twenty people attended including some members of the BAC-STOP team.

The morning of the workshop was spent discussing key issues such as soil acidity, drought etc. and identifying what actions land managers might take to help to address the issues. We used a set of cards to aid the discussions.

We then had a brief exercise to identify whether the actions were likely to be high, medium, or low cost. Unsurprisingly stakeholders outlined that this depended on the context of the oak trees. Finally, we discussed whether a decision tree/framework might be useful and what it might include. Stakeholders suggested that a framework could be useful however it would need to be fairly simple, focus on oaks in different contexts, and be effectively communicated so that it was well used. A report of the workshop has been produced and will be made available to the stakeholders for comment.

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Above: Sandra Denman, Forest Research's Oak Stakeholder's Day 2023

Demystifying Masting, a PhD thesis on the drivers of oak masting in the UK

Ryan McClory, University of Reading

Acorn crops are inconsistent from year to year, for example since 2020 at Wytham Woods just outside Oxford, there has been one large acorn crop, followed by a complete crop failure and then two mediocre years (Fig 1). This quasi periodic and temporally synchronous production of acorns may seem strange but it is an evolved strategy of acorn reproduction praticed by oaks called Masting.

Masting occurs commonly in several taxa of perennial (long lived) plants and is defined as a somewhat periodic and synchronous production of seeds across large areas. So, in 2020 good acorn crops were seen across the UK, similarly during 2021 there were very few sites that produced any acorns at all. Masting in oaks is widely thought to provide an evolutionary advantage for their acorn's survival. This occurs due to a parallel cycle of seed predator satiation and starvation. When there is a bountiful acorn crop ecosystems receive a great boom of resources that will be felt across trophic levels. For example, we may see a direct increase of animal populations that rely on seeds, nuts and berries as a large part of their diet (I.e. the Eurasian jay, *Garrulus glandarius* and the Grey Squirrel, *Sciurus carolinensis*). Increases in these animal populations can indirectly lead to an increased number of ticks (*Ixodes* sp.) that use these small animals as a host, not to mention insects such as the Oak Gall Wasp, (*Andricus quercuscalicis*), which use acorns for reproduction. However, this good year of acorn

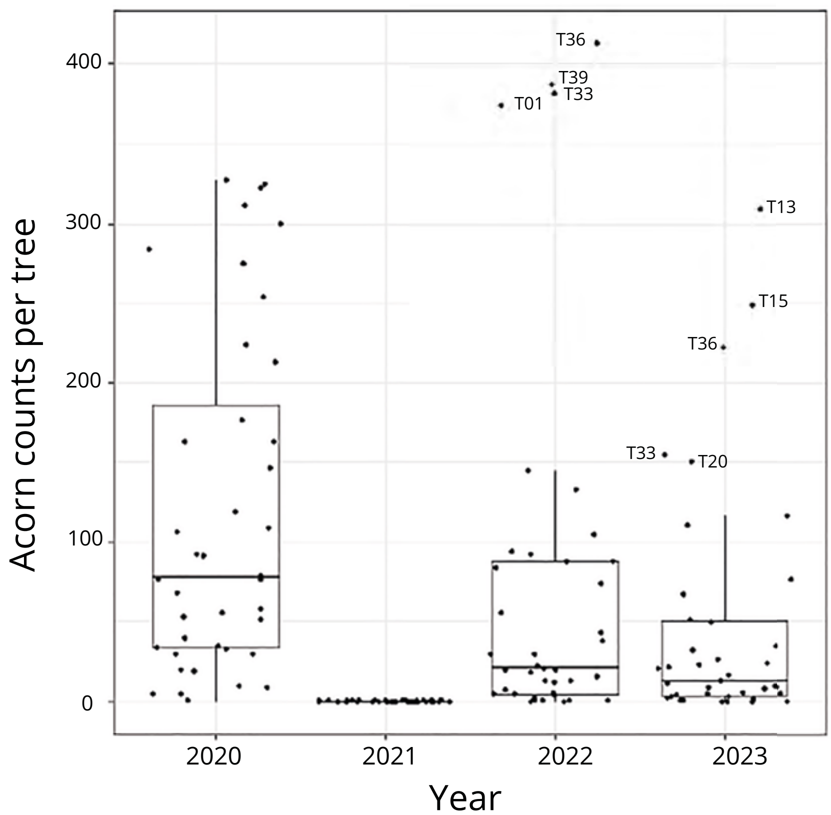

Figure 1: The variation in mature acorn production among 39 oak trees each year at Wytham Woods, Oxford, counted from visual survey (see Koenig et al., 1994). The vertical boxes and bars represent the minimum, 1st quartile, median, 3rd quartile, and maximum values for each year. Individual trees are represented by black dots. Outliers were detected in 2022 and 2023 and are labelled with their tree ID code.

Figure 2: Oak branches on the canopy Walkway at Wytham Woods, Oxford. White bags on the branches are pollen bags (pbsinternational, Scarborough), used to isolate branches from environmental pollen.

Figure 3: Reproductive material collected from the Free Air Carbon Environment site at Mill Haft, Stafford. Maintained by the team at the Birmingham Institute of Forest Research (BIFoR).

production is oft followed by several lean years or even crop failures. The same fauna that took advantage of the larger acorn crop the previous year instead find their population limited and starved of food resources. Consequently, when oaks again produce a much larger crop, low numbers of 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.

This same mechanism that starves seed predators of a food resource is also starving the seed sourcing industry and land managers of a consistent supply of acorns for planting. For instance, due to a poor crop in 2021 (combined with the inability to store acorns and maintain their viability) the forestry commission suggested moving away from using oaks for the 2022/23 planting season. If oaks are to maintain their importance within British Broad-leaved woodlands, we need to understand how masting occurs and how it will be affected by future climate change.

This PhD research into oak masting has examined the problem across multiple scales that include work across five separate studies. Study one has examined how individual trees may vary in their acorn output, finding some individuals to be super producers which in general are the late flowering individuals situated on heavy clay soils and with a more open canopy. Study two attempted to use machine learning to identify weather cues important for oak masting. It was not able to be used to predict acorn crops accurately, however, it did identify that acorn production between sites became less synchronised and even negatively correlated

Fig 4. Oak saplings ready to be transplanted to the field site at Reading University for study 5, a Europe wide common garden experiment organised as part of the PEN-CAFoRR EU COST Action

when the distance between sites was large enough and identified some important variables such the temperature and precipitation in Spring. Study three was the first to identify the presence of a maternal choice in pollen source via the selective early abortion of oak flowers. Oak flowers that were pollinated with "Out of Stand" pollen produced a higher and more consistent acorn crop than those pollinated with pollen collected from within the experimental studies population. This raised interesting questions on the possibility of pollen supplementation to increase supply.

Finally, study four and study five were two collaborations. The first with the Birmingham Institute of Forest Research (BIFoR), counts were made of the number of acorns collected within the Free Air Carbon Enrichment (FACE) site at Mill Haft, Stafford. And comparisons made between the elevated CO_2 array (550 ppm) and the ambient arrays (407 ppm). The study found elevated CO_2 did increase acorn counts, but only when including acorns that had been attacked by predators, highlighting the need to study both when investigating the affects of climate change. Study five was a comparison between planting techniques for forest regeneration. The study compared directly seeding an acorn and allowing it to germinate and grow naturally versus pre-growing the acorns into saplings before transplanting them after the first season. At the site at Reading University there was no noticeable difference in size of oak seedlings after 3 years, but as the full study involves 93 sites across Europe and 12 species of the genus *Quercus* more findings are expected soon.

All of these studies have been fully written up in thesis form within this PhD research, and currently two articles have been accepted for publication in the Journal of Forest Research, the pollination study at Wytham Woods and the collaboration between the Team at BIFOR.

Does susceptibility to acute oak decline have a genetic basis?

Dr Rômulo Carleial, Post-Doctoral Research Associate
Royal Botanic Gardens, Kew

Oaks across Europe have been suffering from decline syndromes at least since the 90s. In Britain, acute oak decline (AOD) has affected thousands of trees, some of which die each year. AOD is mainly characterised by crown dieback and the presence of numerous dark bleeds in the bark, a consequence of necrotic tissue. Many Defra funded studies, some associated with Action Oak, have sought to understand the predisposing factors behind AOD, with many biotic and abiotic risk factors now identified. For example, work done by Forest Research has elucidated the roles of drought and damage caused by larvae from the jewel beetle (*Agrilus biguttatus*) in weakening trees, and the necrotic potential of different species of bacteria often present in the wounds of affected trees. Evidence is building up for the existence of complex interactions between these many factors that in isolation may be insufficient to explain the occurrence of AOD.

The monumental effort aimed at elucidating the environmental causes of AOD has not directly investigated the degree to which there is a genetic basis for predisposition or resistance to this complex syndrome. At Kew Gardens, in collaboration with Forest Research, we are presently attempting to fill this knowledge gap by studying the genomes of almost 1500 pedunculate oak (*Quercus robur*) individuals. Genomes harbour variation in the nucleotides (i.e. A, C, T & G) that compose them, and these variants may on occasion affect the workings of genes. For example, a change in the DNA sequence of a gene may lead to a defective protein product with impaired function. The most common genome variants are known by the mouthful term “single-nucleotide polymorphisms” (SNPs), which are changes in a single DNA “letter”. Our work aims at characterising SNPs across the oak genome and identifying whether some of these SNPs are systematically associated with AOD. If associations are found, we can in principle attempt to identify which genes are being affected by the SNPs, and whether they are related to functions which may credibly be involved with abiotic or biotic stress resistance. We can also use genetic data to inform breeding programs aimed at rescuing populations under threat, for example by predicting the genetic merit – in this case resistance to AOD – of individuals for which phenotypes are unknown (e.g. seeds). Given the multifactorial nature of AOD, we expect genetic resistance – if any – to be driven by the combination of small effects across many genes (i.e. polygenic trait). Precisely because effects are likely small, sample sizes required for statistical analyses must be very large, hence our dataset being one of the largest ever collected for forest trees. This was only made possible by generous funding from Defra over several years, via the Future Proofing Plant Health scheme and more recently the Centre for Forest Protection. This has included a large sampling effort across more than 20 UK sites executed by Forest Research, as well as genome sequencing and analysis conducted at Kew.

Irrespective of whether we find variants associated with AOD or not, our results will be informative. Absence of a link between genetics and AOD susceptibility will suggest AOD is mostly driven by environmental factors so management of the tree micro-environment will be required to mitigate negative effects. Preliminary data from our work seem to confirm the expectation that AOD resistance is polygenic and weak, pointing to environmental factors (e.g. temperature) as the principal causes behind AOD susceptibility. This is worrying since temperatures are predicted to increase over the following decades, which may increase the range of AOD. Our preliminary results will be presented at the International Union of Forest Research Organizations (IUFRO) conference in Sweden this coming summer, where we expect to receive many interesting feedback and suggestions, which should help fine-tune our analyses. We are hoping to finalise the bulk of this research before the end of the year, so stay tuned for our updated results.

Opposite: Sarah, Arboricultural Association, Welsh Government and Dr. Andrew Hacket-Pain at an Action Oak sub-committee meeting

Below: AOD affected tree in Richmond Park, London.

Acknowledgements

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

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Action Oak

Safeguarding survival

Action Oak supports the vital research and monitoring needed to understand better 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 through 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 impact and their ecological impact. What would the loss of such a prominent tree mean for the environments and people who rely on them?

This question isn't just hypothetical; the threat to oak trees has grown considerably. With existing & emerging pests, diseases & human intervention expedited by the climate emergency, the time for action has never been more apparent.

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 critical 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

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