Acute Oak Decline (AOD)

Newsletter

April 2019

WOODLAND HERITAGE
Celebrating 25 years

Forest Research

A Golden Decade of Research for a Silver Anniversary

Sandra Denman, Forest Research

I first encountered and began work on diseased Oak with dark, weeping stem patches, which we now know as Acute Oak Decline (AOD) in the spring of 2008. Later that year I met Peter Goodwin, and found out about Woodland Heritage. Rod Pass (forestry consultant) arranged a visit to one of Peter's woodlands that had an alarmingly high number of prime Oak with stem bleeds. Many trees were dying. At that stage I had no funding for the work that I had begun earlier on the disease, rather the project had actually been shut down because there were many pressing tree diseases vying for funding, and who could forget, times were hard as the great stock market crash of 2008 had happened. Looking back on my 2009 Newsletter I am struck at just how much of the following decade of research is attributable to the ardent determination and commitment of 'the voices of industry', with Peter Goodwin being the unsurpassed champion of the cause, but also so many other fantastic advocates supporting us and our work. Without this support, the past decade of acquiring new knowledge on AOD causes and management, as well as insights into the drivers undermining the health of Oak, would not have been made. In truth it is all of you, our supporters and dedicated quercophiles, who are the true heroes in enabling us to obtain the knowledge that will help safeguard our Oak for future generations.

Sandra Denman, Forest Research

I would have to write pages and pages were I to outline all the knowledge we have gained over this golden decade of research. Instead I have made a summary list of key scientific understandings we have made on AOD, resulting from the support we have received. These also demonstrate the systematic scientific journey we are travelling. Critical knowledge acquired about AOD includes:

- Recognition and definition of AOD as a distinctive condition within the wider Oak decline complex [15]
- Evidence showing that bacteria are the dominant and consistently occurring microorganisms in the stem lesions characteristic of AOD [25,27]
- Formal identification of 15 or more bacterial species new to science, providing names for novel bacterial species, and importantly, for those causing tissue necrosis in AOD [6,7,8,10,11,12,19,30]
- Development of two different rapid diagnostic tools for laboratory use in detection of key bacterial species implicated in lesion formation [22.23]
- Proof of bacterial pathogenicity genes and gene products (e.g. enzymes and toxins) in AOD field lesions indicating a bacterial cause to tissue rotting [36]
- Evidence, using whole genome sequencing (WGS), showing the pathogenic potential of the key bacterial species isolated from AOD disease material (largely *Brenneria goodwinii* and *Gibbsiella quercinecans*, but including *Rahnella victoriana* and *Lonsdalea britannica*), providing the first functional link between the disease forming capability of these bacteria and tissue rot in the field [47]
- Evidence, from laboratory assays and log tests, showing

activation of the pathogenic potential of the above bacteria, and expression of their pathogenicity gene products in controlled tests, that match those found in AOD field lesions. Furthermore, that in laboratory tests, lesions are bigger and closely approximate field lesions when hatching eggs of *Agilus biguttatus* are included. This work has provided highly compelling substantiation that the bacteria, primarily *B. goodwinii*, are the cause of AOD tissue necrosis, but also showed amplification of microbial damage when *Agrilus* beetles were included in testing, and the bacteria could be detected in the larval galleries suggesting that larvae could spread bacteria within trees [36,41,47]

- Field evidence has been acquired of almost 100 % co-occurrence of larval galleries of *A. biguttatus* in close proximity to the lesions in AOD cases we studied – posing the question of whether the beetle has a role in the cause of the lesions or is merely co-incidental to the disease [20,31]
- Knowhow on rearing *A. biguttatus* through its entire life cycle, in captivity, and supplying beetles, eggs and larvae for other aspects of AOD research [38]
- Determining critical temperatures limiting the growth and development of *A. biguttatus* leading to predictive mapping of the distribution of AOD and *Agrilus*, and indicating at risk areas for AOD spread [45].
- Discovery of volatile chemicals (called semiochemicals, which are naturally occurring, beetle behaviour-modifying chemicals) that serve as attractive volatile cues from stressed trees and bacteria, for *A. biguttatus*. These discoveries will lead to development of various trapping methods for control if required [29]
- Determining, mapping and monitoring AOD distribution across England and Wales based on a designed national survey and use of citizen science reports made to FR Alice Holt. [32]
- Demonstrating significant correlations of AOD occurrence with landscape scale predisposition drivers: AOD is more likely to occur in areas with low rainfall, high temperature and low elevation sites, and with high dry nitrogen deposits on leaf surfaces, and low dry (atmospheric) sulphur content. [42]
- Developing an AOD risk map. [42]
- Uncovering a spatial pattern to within site occurrence of AOD where affected trees occur in groups and tree to tree spread occurs locally. Newly bleeding trees typically cluster around trees with old bleeds, but also around trees with exit holes, suggesting outward disease spread. Annual fluctuations in disease occur, and more than 1% of symptomatic trees die each year, while around 40% of symptomatic trees go into remission [24]

Research on the distribution and spread of AOD at the beginning of the project gave us confidence about where the disease occurred in the UK and of the dominant influence of biotic factors in the diagnostic symptoms. It also identified a new northern boundary to the occurrence of AOD which, with modelling, has been correlated with the thermal requirements of *A. biguttatus*. Analysing national datasets and modelling using environmental parameters has identified major predisposition factors driving the disease but also identified those limiting it. Areas at higher risk of getting AOD are in the warmer drier parts of the country with higher nitrogen pollutants than those in cooler, wetter parts. Our research has also shown for the first time that the stem bleeding and tissue rot characteristic of AOD is attributable to a polybacterial pathobiome (disease complex) and that inclusion of *Agrilus* amplifies damaging effects. It is clear that more work is required on the specific roles and mechanisms involved in predisposition of Oak, and whether or not there is a genetic basis to susceptibility to AOD as well as understanding the precise role of *Agrilus* in the disease.

To this end we have been really fortunate to receive another £700,000 grant from Defra and have been able to mesh the research carried out under the FPPH (Future Proofing Plant Health) Oak Resilience scheme, with a number of Woodland Heritage funded projects. In the second half of this News Letter we tell you more about the various research strands and the exciting discoveries we are making. This unique public-private partnership is enabling us to create a truly integrated team, carrying out dedicated, detailed analyses on well characterised and monitored research sites, and creating a database and infrastructure for long term monitoring. These are the essential fundamentals of understanding tree health and management over the long term. There is no doubt that the acquisition of field evidence is giving us a great start to developing holistic insights into what constitutes a healthy Oak tree, and the drivers behind declining Oaks.

However, I also wanted to give a wider perspective of the significance of AOD research carried out to date. As you probably know, a number of the bacterial species involved in AOD are closely related to well known, very damaging tree pathogens. For example, *Brenneria goodwinii* is related to *B. salicis* the causal agent of 'water mark' disease of willow, which was first detected in cricket bat willow plantations (*Salix alba* var. *caerulea*) in eastern England. It is also related to *B. nigrifluens* and *B. rubifaciens*, the bacteria that cause shallow and deep bark canker diseases respectively, on walnuts. Another example is *Lonsdalea*

Drippy blight on Quercus rubra *in Denver. Note the lesions on the twigs and in the inner tissues (red arrow)*

Drippy blight researchers: Dr Rachel Sitz and Dr Ian Pearse, Denver, USA

britannica found in some AOD lesions, which is closely related to *L. quercina* a very serious pathogen of acorns and now emerging as a twig canker disease on Oak in the USA, called Drippy Blight. The HRM rapid diagnostic tool (see p7) that Viky has developed is so accurate and sensitive, that it could be used to detect closely related plant pathogens, and so the tool has global application, especially to plant health services in monitoring imports. Similarly the taxonomic clarity that Carrie has brought to the polyphyletic (mixed) bacterial genera has far reaching application across medical, veterinary, plant and industrial fields. Furthermore, although the medical research field must be credited with being the harbingers of the concept of polybacterial disease causation, we have been among the first to demonstrate this in trees, producing a template for further research on complex tree diseases using the most modern biological technologies. We have had to be bold in challenging the traditional model of one pathogen = one disease in trees, as James Doonan elaborates later in this journal. We are therefore breaking new scientific conceptual grounds, which pave the way for future generations to understand complex plant health systems. Over the past decade we have achieved the highest level of professional, scientific training, grounded in robust scientific methodology and critical appraisal, thus creating new generations of forest health scientists. Woodland Heritage has funded 14 PhD and 11 Post-Doctoral students and given work opportunities to youngsters and mature graduates and assistants that may have been away from the work place for a time. Defra has also helped in this regard, so through these funding and training collaborations, we can be proud of the number of forest health scientists trained. Furthermore, we know that AOD occurs more widely in continental Europe, although not formally recognised under the name AOD. Our research has, for the first time, provided the evidence and uncovered the cause of the stem bleeds. We now carry out swab tests from trees in Europe and give help and training to European plant health officers when asked.

It seems that 2019 is a special year, special birthdays and/or anniversaries and so there is much to reflect on and celebrate. This year is the centenary of the Forestry Commission, and the Silver Jubilee of Woodland Heritage. Looking back over the past decade of research I cannot help feeling that our work has been a job well done, whilst looking forward to the next decade of research I am so excited, because I can see that the hard-won early foundations will now translate into practical management applications, not only to counter AOD, but for the promotion of good health and resilience in Britain's Oak populations and more generally for its trees. I once got a marvellous card with a picture of a big old Oak tree. It said: "It takes a long time to grow an old friend." So too does it take a long time, and patient, persistent effort to carry out the research needed to understand the whole of the trees that we love so much. We could not carry out the research that is helping us understand how Oak trees function as a whole, leading to better management for resilience and long-term survival, without the sustained support from people passionate about the future of our trees, and the unabated enthusiasm and dedication of the scientists. Thank you Woodland Heritage, thank you Defra and the Forestry Commission, and special thanks to the other charities and benefactors, who donate funding for our research through Woodland Heritage. Thank you for believing in us and for supporting us. We plan to continue delivering high quality science and solutions over the next ten years with your continued, designated, kind and generous support.

A taxonomist's timeline of AOD

Carrie Brady, Post-Doctoral Research Fellow at the University of the West of England

I can still clearly remember the day I first heard about the decline threatening Oak in the UK. It was early 2008, I had just completed my PhD at the University of Pretoria (UP) in South Africa and was experiencing a lull before my first post-doctoral research position officially started. My supervisor (Prof. Teresa Coutinho) told me her friend, Sandra Denman from Forest Research (FR) in the UK, would be visiting UP and needed help to identify bacteria isolated from diseased Oak. As a bacterial taxonomist (someone who identifies and classifies new bacterial species) specialising in plant pathogenic bacteria, my interest was immediately piqued. Sandra and I spent a hectic few days performing DNA sequencing on the bacterial cultures she brought with her. From our very first results, it was immediately clear that the organisms causing the symptoms on Oak did not belong to one single bacterial species. Feeling a bit disappointed that we weren't able to conclusively identify the bacterial isolates, Sandra returned to the UK while I promised to refine the analysis on the DNA sequences.

Over the next year, Sandra visited UP several times, bringing more strains from an increasing number of symptomatic Oak sites. With each visit we inched closer to understanding that there were at least two different genera and four different species among the isolated bacteria, all of them new to science. However, to (officially) describe and classify these bacteria, tests and assays needed to be done which couldn't be performed at UP at that time. In 2009, I was awarded a postdoctoral grant to work with bacterial taxonomy experts at the LMG Bacteria Collection in Gent, Belgium. My project was to focus on the taxonomy of plant pathogenic *Enterobacteriaceae*, and as luck would have it, the bacteria isolated from Oak belonged to this family. I was able to incorporate the Oak isolates into my new project and in 2010, *Gibbsiella quercinecans* was described, followed by *Brenneria goodwinii* and *Lonsdalea britannica* in 2012.

When my grant in Belgium ended, I had hoped to join Sandra at FR, but it seemed that it wasn't meant to be.

Victoria Bueno-Gonzalez and Carrie Brady

The financial crisis had taken hold and research funding was virtually non-existent in 2011. Sandra and I had been exchanging despondent emails and phone calls for months when finally, there was good news. A charity called Woodland Heritage understood the importance of research into Acute Oak Decline, and it wanted to help. It took several months, but in an incredible feat of fundraising by people passionate about saving the Oak, enough money was raised to fund my research for six months. By this time, my husband and I had moved to Bristol and commuting to FR in Hampshire wasn't possible. With Sandra's encouragement I looked into universities in the Bristol area and found that Dawn Arnold (a prominent molecular plant pathologist) was based at the University of the West of England (UWE). Dawn agreed to host me in her lab for six months while I continued the taxonomic descriptions of the increasing number of bacteria that were being isolated from symptomatic Oak across Britain.

Seven years and two maternity leave stints later, I am still here at UWE! With the continued generous support from Woodland Heritage, I've been able to describe a further seven species belonging to the genera *Gibbsiella*, *Brenneria* and *Rahnella* as well as to begin investigations into the pathogenic relationship between these bacteria. I have also been given the opportunity recently to co-supervise Victoria Bueno-Gonzalez, the first PhD student to be jointly funded by Woodland Heritage and UWE.

She has developed a rapid identification technique, which drastically reduces the time it takes to detect accurately if the AOD-associated bacteria are present in symptomatic Oak. Victoria is also in the process of describing several new species of *Pseudomonas* which are commonly isolated from Oak trees. The process of describing novel species has changed steadily over the last ten years, incorporating more accurate and rapid methods to improve bacterial classification.

Being involved in the AOD project from its infancy has allowed me to refine my taxonomic skills and gain knowledge and experience with each new description paper which I am now able to pass on to a new generation of bacterial taxonomists. It's an exciting time to specialise in taxonomy, especially with so much still to discover about the bacterial community of Oak, from the endosphere to the rhizosphere. I am grateful to Woodland Heritage, especially the late Peter Goodwin who I had the pleasure of meeting several times, for giving me the opportunity to be a part of this journey from the very beginning.

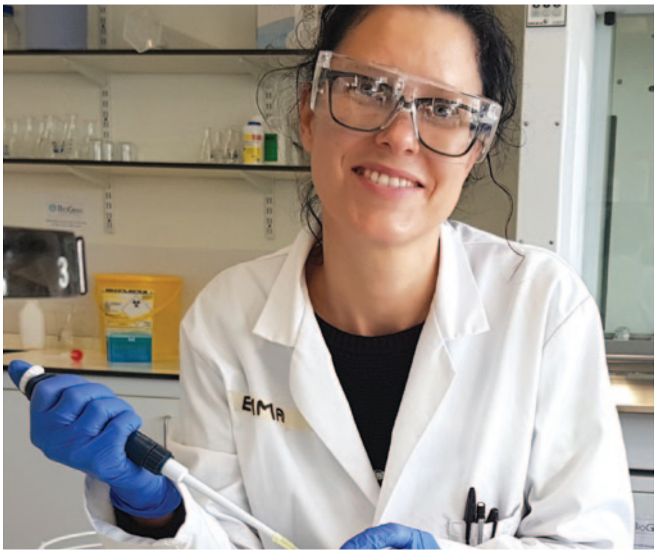
Potential transmission pathways of *G. quercinecans* and *B. goodwinii*

Emma Bonham (PhD student, Harper Adams University)

This project is now in the final year of investigating how the bacteria associated with AOD could be moved to new hosts. As an ecologist I have been able to apply my existing skills to this project and it has been a mixed three years of highs and lows. It has involved collecting insects, leaves, acorns and rain at various sites in England. After a long field season plenty of lab analysis meant I had the opportunity to learn a new skill set including pathology and microbiology.

National Trust field site, Shropshire

So far positive detections of *G. quercinecans* and *B. goodwinii* on the leaves and acorns of symptomatic and asymptomatic Oaks indicates that the bacteria may be existing as an epiphyte on external Oak tissues. To date there has been no finding of the bacteria on collected insects but analyses are ongoing. The findings reveal potential modes of transmission for the bacteria to be moved to new hosts via rain splash, air or insect herbivory. Entry pathways into the tree may therefore be via leaf openings or damaged tissues. Further sample processing for the bacteria and results analysis is a priority for the coming months.



Emma Bonham, (PhD Student, Harper Adams)

I gratefully acknowledge the support from Woodland Heritage for funding this PhD. I am thankful for the learning opportunities it has provided me to develop as a researcher.

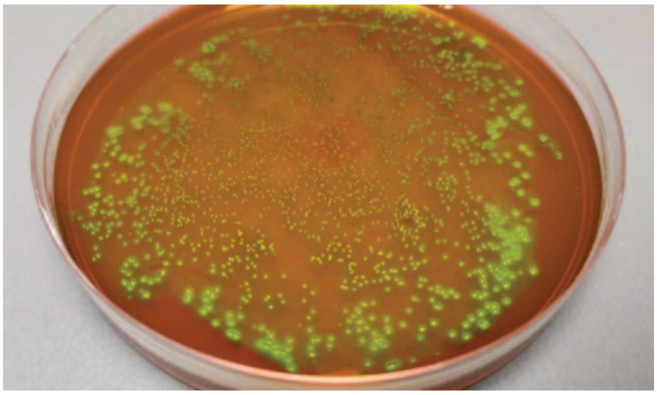
Being part of a new generation of researchers in AOD

Victoria Bueno-Gonzalez, PhD student at the University of the West of England (Bristol)

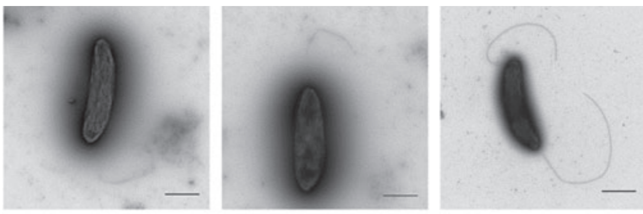
PhD project title: **Towards a rapid diagnostic method to identify bacteria associated with AOD**

Having had an interest in environmental conservation for a long time, I was honoured to receive a PhD studentship co-funded by Woodland Heritage and UWE, working on Acute Oak Decline (AOD). I am a biologist with a keen interest in biodiversity conservation. After obtaining my MSc in Biodiversity Conservation from the University of Salamanca in Spain I moved to the UK following a dream of further developing my career in this field of the sciences. I began work as a laboratory technician at the University of Leeds. This position allowed me to improve my practical skills in molecular biology which I needed for my next step in my career - obtaining a PhD. My attention was grabbed by an exciting new project working on identifying new bacterial species associated with AOD and developing a diagnostic method for rapidly detecting bacterial disease causing agents that were implicated in AOD which led to death of mighty Oaks. The moment I read the description of the project I knew this was going to be the centre of my life for the next three years.

On this journey of personal and professional growth, I am very lucky to have the exceptional guidance of my supervisors Prof. Dawn Arnold, Dr Sandra Denman, Prof. Joel Allainguillaume and especially Dr Carrie Brady. Thanks to Carrie's expertise, I quickly learned what was necessary to be sure of the precise identity of the bacterial strains we isolated and were working with and that this task involved a lot of detailed work. To identify and properly classify the microbes that we isolated from Oak trees was the foundation for the following task of designing accurate diagnostic methods for specific bacterial species associated with AOD. As a result of three years' hard work, we are in the process of publishing scientific articles describing several new species of bacteria discovered in some AOD lesions, and which now need further evaluation to find out if they contribute to the tissue rotting process in AOD. In addition, and thanks to the continuous support of Woodland Heritage, I am now finalising a diagnostic method which I hope will save time and resources when processing the numerous samples from diseased Oaks that the laboratories of Forest Research receive. The design of the diagnostic method, which is based on the detection of the pathogens' DNA, required months of experimentation and fine-tuning of a cutting-edge technique called high resolution melting analysis. I intend to complete my PhD in the next few months and now my aspiration is to continue to help protecting trees and forests by becoming a forest pathologist with strong bacterial and mycological skills, but also to help to train the next generation of forest pathologists in the same way that I was so fortunate to experience. I am finishing my project with more enthusiasm and way more knowledge than when I started, and I am very proud of being part of this multidisciplinary effort to save the British Oak.



Colonies of Brenneria goodwinii *in EMB culture medium. The colonies are metallic green on this medium*



From left to right: Pseudomonas daroniae, P. dryadiae, P. kirkiae. *Scale: 1µm*

Are slow-growing Oaks more likely to develop AOD?

Using dendrochronology to gain insights into host susceptibility to *Agrilus biguttatus*

Katy Reed, Jack Forster, Sandra Denman, Nathan Brown, Simon Leather, Daegan Inward

After completing my PhD on the role of *Agrilus biguttatus* in AOD, Woodland Heritage has funded a post-doctoral study which has allowed me to continue some important work I started during my PhD: examining the growth of AOD-affected trees using dendrochronology. A tree's stem growth may be used to measure its overall health, and dendrochronology, or the measuring and cross-dating of tree rings, may be used to look for differences in growth rates between trees. The current study, funded by Woodland Heritage, has two main objectives. The first is to determine whether trees that are affected by AOD have grown more slowly throughout their lifespans, or if their growth has slowed recently due to climatic stress such as drought, so predisposing them to the disease. The second is to look at the influence of colonisation by *A. biguttatus* on affected trees' growth and their likelihood of recovery from AOD.

The study is well underway. In total, tree cores were cut from almost 250 trees, across five sites, to try to represent a range of woodlands across England. Four of these sites have been monitored for AOD for a number of years, so the health histories of the trees are well understood. The selected trees represent the full AOD spectrum, from healthy to long-term symptomatic trees, with and without the exit holes of *A. biguttatus*.

Researchers at Forest Research received training from dendrochronology experts at Swansea University in preparing, measuring, and cross-dating the cores. The cores must be dried and mounted on wooden blocks, and sanded with progressively finer sandpaper. The cores are then scanned at high resolution using a large-format scanner, and, on the resulting images, the boundaries of each annual growth ring are marked using special software. The resulting tree "chronologies" are then checked for accuracy by cross-dating first within, and then between sites. For the project, Jack Forster has developed a unique statistical modelling method to look for differences between healthy and affected trees.

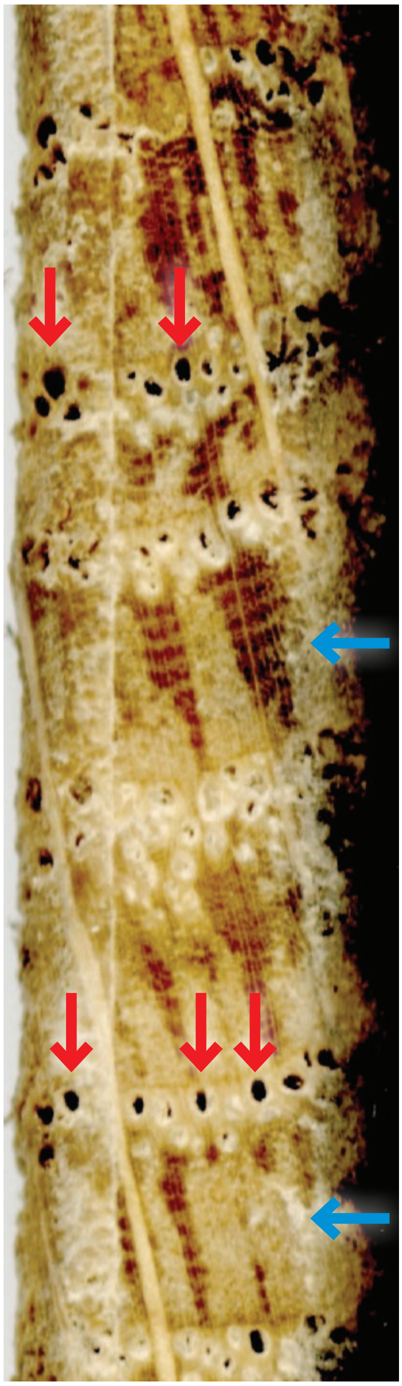
Final results are expected later this year. At some of the sites, preliminary results suggest that trees which had long-term AOD symptoms grew more slowly than healthy trees, throughout their lifespan. If this finding holds true at other sites, it may have implications for management of AOD. Opening up stands by removing the most suppressed trees may reduce the number of trees likely to develop long-term AOD infection, and increase the resources available to the remaining trees. The role of the beetle in AOD would, however, need to be better understood, as opening up stands could increase under-bark temperatures, benefiting beetle development.

The generous support of Woodland Heritage has allowed me to continue this important project, improving our understanding about the past health of trees that develop AOD and the role of *A. biguttatus* in the syndrome. It has also has enabled me to begin a career as a forest entomologist. Training the next generation of scientists in this field is vitally important given the multiple threats trees face, from climate change to invasive pests and pathogens. I hope my work in this field will help preserve these important and fascinating ecosystems for future generations.

Katy Reed at her PhD graduation at Harper Adams University, holding new baby son Miles

Daegan Inward, Senior Entomologist, Supervisor and Principal Investigator on Agrilus *– AOD related research*

(Left) A high resolution scan of an Oak tree core, showing annual ring boundaries, and differing recent growth rates. The outer bark and most recent growth are at the bottom, oldest tree growth rings are at the top



(Aove) A close up of a section of the core. The red arrows point to the very large xylem vessels of the spring or early wood that carry water from tree roots to the leaves. The blue arrows point to the late wood, which is made up of heavily lignified cells that have a structural role

Revealing the semiochemistry behind the ecological links of AOD

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József Vuts, Chemist

Pest insects can have a serious negative impact on forest ecosystems and the economic services and societal benefits they provide. For better detection, monitoring, mass-trapping and biological control of such pests, semiochemicals (naturally occurring behaviour-modifying chemicals) offer a non-toxic, environmentally benign alternative to insecticides. For example, the chemical ecology of the emerald ash borer (EAB), *Agrilus planipennis* (Coleoptera: Buprestidae), has been extensively studied to mitigate the damage it causes in North America. As part of Defra and Woodland Heritage funded collaboration between Rothamsted and FR since 2013, we are investigating the semiochemistry of a native British beetle species, the two-spotted Oak buprestid (*A. bigutattus*) associated with and implicated in aspects of Acute Oak Decline (AOD), and related to EAB.

The biotic component of AOD is characterised by the co-occurrence of necrogenic microbial communities and larval galleries of the herbivore *A. biguttatus* within Oak bark. Our studies identified volatiles from live Oak leaf and bark that *A. biguttatus* beetles found attractive, and which thus likely helps them find trees for feeding, mating and egg-laying.

However, the trees and the beetles do not interact with each other in an isolated environment, but as parts of local ecological communities. For example, the same blends of Oak leaf and bark volatiles may not only be exploited by the woodborer beetles, but also their natural enemies, such as parasitic wasps, that seek host larvae. They eavesdrop into the volatile emissions that accompany the presence of the beetles on the host plant.

Similarly, necrogenic AOD bacteria that co-occur with *A. biguttatus* larvae under the bark may have a significant effect on the beetles' interactions with other organisms within a woodland community. Plant and animal parasites increase their transmission rate by their insect vectors via modifying the smell of their host or by emitting attractive blends of volatiles themselves. We found that mated *A. biguttatus* females respond positively to volatiles from certain AOD bacteria in laboratory behavioural studies, suggesting that the beetles are attracted to the scent of these bacteria in the forest.

The complexity of the chemical ecological network of AOD offers opportunities for further controlled laboratory and field trials to identify key semiochemicals that underline the proposed links between Oak trees, *A. biguttatus* beetles, bacteria and parasitoids.

We are using air entrainment to collect volatiles from Oak trees, *A. biguttatus* and bacteria, coupled gas chromatography-electroantennography to locate bioactive peaks in air entrainment extracts with beetle antennae, gas chromatography-mass spectrometry to identify bioactive compounds, multivariate statistical analyses to uncover chemical patterns linking ecological interactions, and laboratory and field bioassays to determine beetle behaviour in response to odour blends. To this first end, we are conducting field trials in 2019 to optimise an attractant trap for *A. biguttatus*. This research will underpin the development of semiochemical-based tools to mitigate the impact of AOD through the management of one of its key components.

Air entrainment equipment for identifying volatiles for beetle trapping placed high up in tree crowns in the Forest of Dean

A decade of observations documenting changes in declining Oak woodland

Nathan Brown (Rothamsted Research)

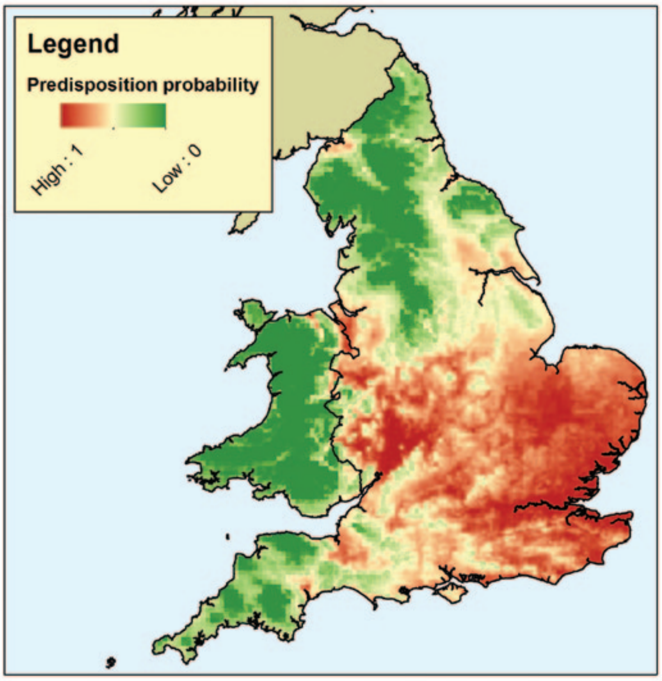
I first met Peter Goodwin when I was a Masters student. He spent an afternoon showing me a woodland that was in decline. He made his point, enthusiastically as ever, that something had to be done. The trees were in poor condition, their stems littered with weeping black stains and no cause had been determined. Shortly after these discussions Woodland Heritage made its first contributions to AOD research. At the same time Forest Research had acquired a new GPS device and thus the ability to map individual trees within woodlands accurately. We established eight monitoring plots in woods and parkland across the country to document changes in condition. The aim of our monitoring was both to understand how AOD symptoms developed over time as well as examining the relationship between AOD and the underlying health of Oak trees. In 2018 we collected the tenth year of observations from these plots.

In only the second year of monitoring, the detailed study plots had already offered great insights into the AOD syndrome. We saw affected trees grouped in clusters, with new infections often occurring around the edges of established groups. This was the first clue that something biotic was causing the stem lesions and gave reassurance that the bacteria isolated from the lesions may be causing the decay.

An even more revealing discovery also quickly became apparent: AOD trees could enter remission, they could form a callus and heal over old lesions. After some initial disbelief and rechecking of records, this finding left me hopeful. Signs of AOD on stems were not necessarily an indicator of imminent death - these *Quercus* trees really are robust! Current research is focused on understanding how to reduce the impact of AOD on trees and woodland. This includes work to reveal how the environment influences decline susceptibility and severity, and studies of the genetic and biochemical pathways that are active in trees which are recovering.

Over the last decade the rates at which new AOD infections occur has fluctuated each year, in almost exact opposition to the rate at which trees heal. In some years we find many more new trees are affected and in some years many more trees heal. Affected trees were most prevalent in 2012- 2013 and again in 2015. Perhaps surprisingly, the hot months last summer did not cause a large jump in the number of affected trees, although it was by far the worst year for mildew and defoliation that we have recorded in our monitoring. It will be very revealing to see the longer-term impact of this extreme weather in the coming seasons, especially if the weather stays dry. We are currently investigating the mechanisms that drive AOD outbreaks focusing on how the number of symptomatic trees correlates with weather patterns.

In the first year of our current, Woodland Heritage funded, project I worked with Elena Vanguelova and Samantha Broadmeadow to analyse the national distribution of AOD affected sites. We tested whether



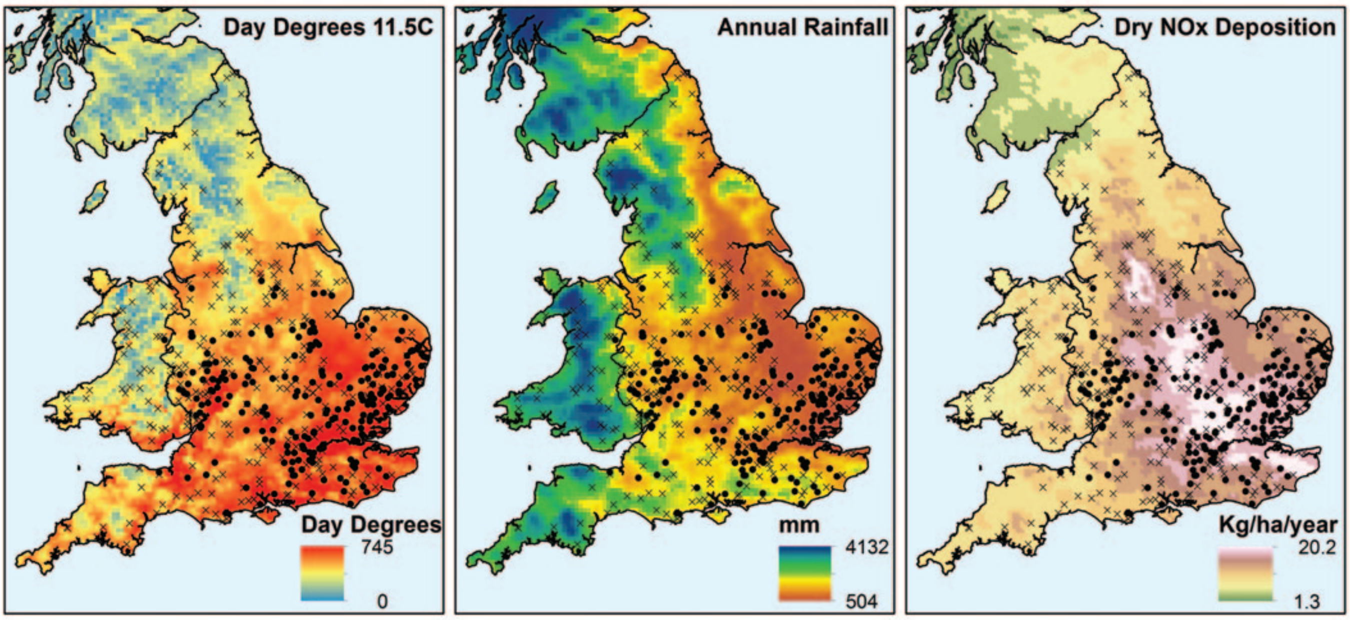
A risk map for the probability of AOD outbreaks based on environmental variables linked to predisposition factors.

Nathan Brown with Mark Ferryman using the Trimble device for mapping trees in woodland

AOD could be influenced by long-term environmental trends. We used all the reports of AOD sent in to FR by landowners and agents, in conjunction with the results of Defra funded surveys in 2013 and 2014. The analysis showed that AOD-affected sites were more likely to be found in the warmest parts of the country and those with low annual rainfall. There were also significant correlations with low elevations and dry high nitrogen (NOx) deposition. These patterns suggest that environmental conditions play an important role in predisposing Oak trees to decline. Essentially, if site conditions are poor the Oak trees will be in a weakened state and more susceptible to AOD. It is our current hypothesis that annual variations in temperature and rainfall will affect the extent of AOD prevalence at our monitoring plots, and thanks to Woodland Heritage's continued support we will be able to test this formally in the coming year.

On a personal note I would like to thank all who have supported Woodland Heritage's fund raising over the last decade. The monitoring plots that this helped establish have been a vital component of my work at FR during my Masters, my PhD and beyond. They still provide crucial data for our current projects and have been used to underpin wider studies by the FR led consortium such as investigating soils and nutrient availability; host metabolomics and genetics; root microbe communities and ectomycorrhizal fungi; Oak phenotypes and dendrochronology. These projects, mostly conducted by students and early career researchers have furthered our understanding of both AOD and Oak decline, while at the same time they have helped to develop a new generation of forest health scientists.

Location of AOD detection in relation to three significant environmental predisposition factors. Black dots represent confirmed AOD affected woodlands. Grey crosses represent symptom free woodlands



Temperature accumulated in degrees per day above 11.5°C. The map represents the strongest temperature trend tested which is linked to the minimum thermal requirement of the Agrilus biguttatus *life cycle*

Average annual rainfall (in mm)

Dry nitrogen is atmospheric nitrogen deposited outside of rainfall. NOx denotes the different types of nitrogen, which include NO, NO_3-, NO_2-

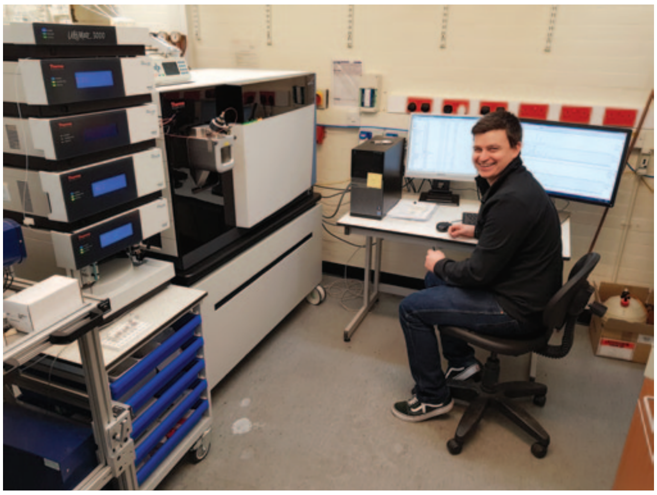
New generation research towards developing predictive tools for monitoring Oak health

Jasen Finch, Manfred Beckman and John Draper (Institute of Biological, Environmental and Rural Sciences (IBERS), Aberystwyth University)

Everyday an Oak tree encounters various environmental stresses such as high levels of pollutants, nutrient imbalances, drought, frost, insect defoliation, pest or disease attack. Although we cannot see it, the tree will be taking active defensive measures. For example, if there is a water shortage, leaves may close their stomata, which are tiny openings on their undersides, to reduce water loss. Or if hungry insects chew on leaves they produce toxic chemicals to make them inedible. However, these defences come at an energy cost to the tree, and deflecting resources into defence means that less vital sugars and energy will be available to maintain growth and vigour.

In the short term these stresses have little effect on a healthy Oak tree as it has vast reserves of carbohydrates stored in the sapwood of its trunk ready to be mobilised to produce extra energy when needed. In the longer term however, if these stresses occur for prolonged periods over a number of growing seasons, the stored energy reserves will become depleted and the trees less able to defend themselves. Furthermore with depleted reserves, there will be less available energy for spring bud burst and maintenance of the crown and the woody layers that the tree uses for transporting water and nutrients. This leads to a reduction in crown condition, dieback and decline. Weakened trees will also become more susceptible to pests and pathogens such as the *Agrilus* beetle and bacteria associated with Acute Oak Decline (AOD). Predisposing stresses involved in Oak decline are expected to become increasingly prevalent with the onset of climate change.

Oak growth and defences are products of complex networks of chemicals and chemical reactions, encoded in the tree genes, and activated by the interaction between the trees and the environment. The Aberystwyth University team (helped by funding from Woodland Heritage) are using modern techniques to measure hundreds of chemicals within Oak tissues, requiring only a small bark punch sample about 5cm in length and no bigger in diameter than a garden pea, to get an idea of the metabolic processes within healthy and diseased Oak trees. These samples are analysed searching for patterns that will distinctively characterise and separate a declining Oak tree from a healthy tree, and also patterns that could signify the potential to decline in the future.



Jasen Finch using the high resolution mass spectrometer for identifying and measuring chemicals within Oak wood samples

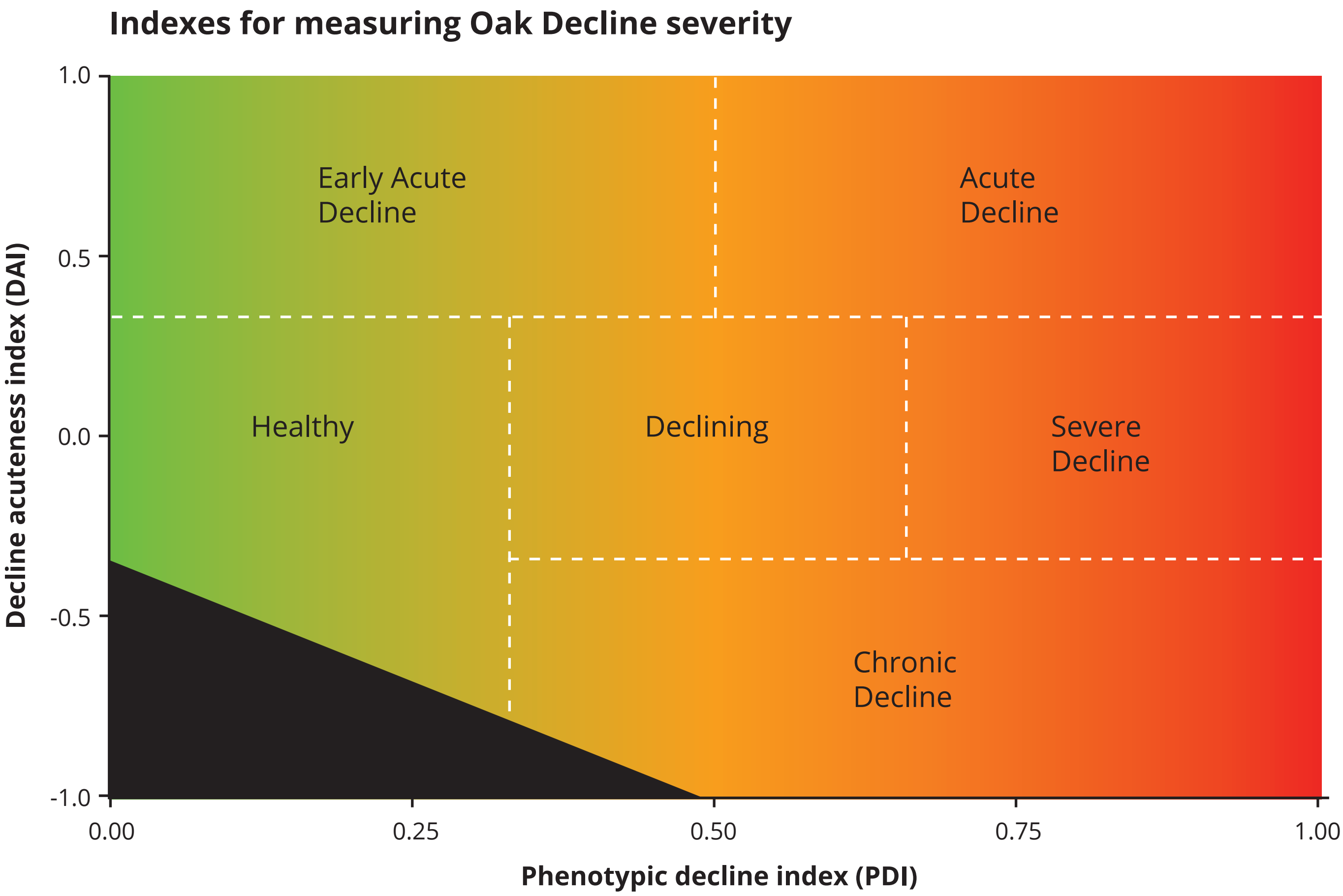
During this search it became apparent that a sensitive, accurate method of visually rating tree health condition was an essential pre-requisite to the metabolic analyses. This is called phenotyping Oak trees and it measures what the trees look like, giving a health condition rating. We used a range of characteristics such as size, shape, crown condition and volume, and the presence of particular insects, fungi and bacterial species. To accurately capture the real world spectrum of decline severity we developed two measures combining all the different characteristics that reflect this spectrum of decline. The first measure we call the Phenotypic Decline Index (PDI), which provides a measure of how severely a tree is declining and is mainly influenced by crown size and condition.

The second is the Decline Acuteness Index (DAI) that allows differentiation between acutely declining Oaks (AOD), chronically declining Oaks (COD) and those that suffer both conditions simultaneously. The characteristics used are easily measurable in the field and require no specialist equipment. They allow comparisons to be made for the same tree between years as well as comparisons of trees between different locations. These could have great utility in forest health monitoring for both forestry management and research.

Using samples collected from both healthy and COD trees from Chestnuts Wood in the Forest of Dean in September 2016 we identified chemical patterns diagnostic of the decline within these trees, and were very excited by the clear chemical distinctions between healthy and diseased trees. Nevertheless we knew that we had to do further sampling and testing to gain more confidence that our results would be valid over a wider sample. Excitingly, using further samples collected in 2017, we were able to confirm that these patterns remained stable between years.

We were then also able to back test the method by taking bark punches from an additional 40 trees that had not yet had their health condition characterised, and ran the chemical finger prints of the samples and predicted the health status of the trees with 70% accuracy. These findings are the first confirmation that the chemical composition of living cells within the outer woody layers provide an indication of tree health condition, but also, that it has wider predictive capability.

Working with Nathan Brown and the FR team led by Sandra Denman, we have developed a statistically robust method of visually characterising tree health condition (phenotyping) that may be used by the wider forestry community, and we have discovered diagnostic metabolic indicators of these tree health conditions. Further research is now required to confirm these results at other sites across the UK and wider afield. From this research, it is hoped that practical tools for accurately measuring and monitoring Oak health can be developed, useful and accessible for the whole forestry community from woodland owners to foresters and researchers.



Visual index measures for Oak Decline severity: Phenotypic Decline Index (PDI) is measure of Oak Decline severity. Decline Acuteness Index (DAI) is a measure that allows the distinction between chronic and Acute Oak Declines. Dashed lines show boundaries between decline statuses

The identification, ecology and function of *Armillaria* species on Oaks in Chestnut Wood, Forest of Dean

Mallory Diggens (PhD Student), Bangor University

Mallory Diggens[1] Sandra Denman[2], Mike Hale[1] and James E. McDonald[1]

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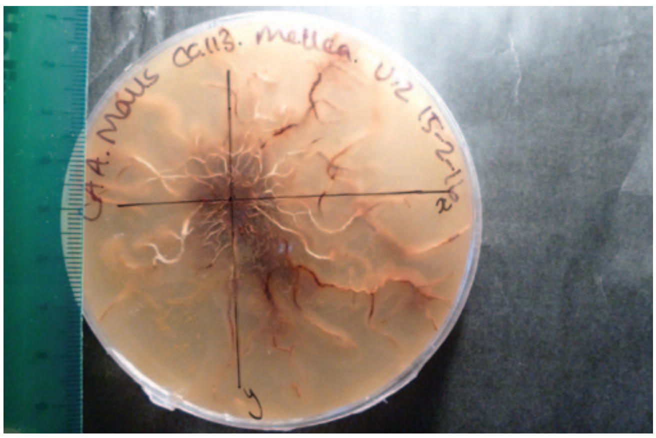
2 Forest Research, Centre for Ecosystems, Society and Biosecurity, Alice Holt Lodge, Farnham, Surrey GU10 4LH, UK

The role of *Armillaria* and root health is becoming a vital part of understanding tree declines across the UK. For many years *A. mellea* has been identified as an important pathogen of trees and woody shrubs particularly in gardens across the UK, whereas our research is focussed on plantation Oak and highlights that *A. gallica* is more important within Oak forest stands. This species is known to behave either as a pathogen or as a saprophyte, and developing an understanding of when or how its behaviour switches is an important factor in understanding infection and disease progression. I began a PhD in 2015 investigating *A. gallica* on a 65 year-old Oak stand at Chestnuts Wood in the Forest of Dean that had a serious outbreak of buttress root and collar rot that caused high levels of mortality.

The PhD predominantly looks at the ecology and role of *Armillaria* on a site affected with Chronic Oak Decline (COD). Two site visits were completed. The first site visit was to gain information on the ecology of *Armillaria* across the site, specifically addressing the question: are there correlations between site soil factors and the presence and severity of *Armillaria* colonisation? Wood panels, rhizomorphs ("bootlaces" of the fungus) and soil samples were collected. *Armillaria* isolates were cultured from the wood panels and rhizomorphs, and soil moisture content, pH, phosphates and nitrate levels were measured. Only *A. gallica* was isolated from the site and the soil analysis highlighted soil moisture content as significantly different between symptomatic and healthy trees, where there was higher moisture in soil around diseased trees than healthy ones suggesting that root systems of symptomatic trees are less able to take up water. The second site visit was to infer the role of *Armillaria* during infection by assessing gene activity comparing infection in healthy trees with that in diseased ones. This sequencing data will give a snapshot of both the tree and *Armillaria* gene activity which will lead to insights about function and host response. The diseased trees were identified by reduced crown, the presence of lesions and rhizomorph growth. Healthy trees chosen showed no signs of infection and no rhizomorph growth at the collar. Wood panels were collected from diseased and healthy trees, snap frozen and the RNA isolated and sequenced. Hopefully we will be able to improve our understanding of what triggers a change in the behavioural mode of *A. gallica* and this maverick fungus' functions (i.e. mechanisms) in host roots. This information will underpin appropriate management guidance to avoid losses due to *A. gallica*.

I would like to thank Woodland Heritage for continued support throughout this time. It has given me the opportunity to develop the work on *Armillaria* ecology at a site and tree level, but also to give data on its functionality as a pathogen.



An Armillaria *isolate grown on carrot agar*

It takes a long time to grow a research programme

These pictures remind us of our roots 2008-2009, developing to branching out in more recent times 2016-2019

Nathan Brown removing his first panel from a tree 2009

Bob Bellis and tree climber getting to the top of the bleeds 2008

Daegan Inward and Nathan Brown examining trees in Bedford 2009

Andrew Jeeves and Susan Kirk reducing Oak panels to a laboratory manageable size 2008

Information notice Peter Goodwin placed in Winding Wood

Peter Goodwin and Rod Pass looking at AOD in East Anglia 2009

Nigel Straw – principal entomologist at FR looking at bleed on Oak in the Forest of Dean 2008

Dave Wainhouse (senior entomologist) examining AOD bleeds in Wyre Forest 2009

Rod Pass examines a severely AOD diseased Oak 2009

Andrew Falcon sampling an AOD lesion using traditional chisel and mallet 2016

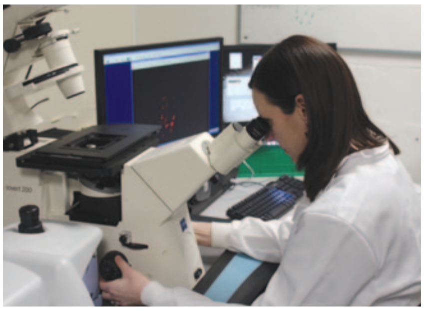
Elena Vanguelova sampling soils to establish whether there are links between AOD and soil properties 2016

Richard Buggs using multiple computer screens in genome assembly and analysis 2019

Lawrence Scott of Summer Isle Films filming foliage sampling at Bigwood 2016

Air entrainment capturing volatile emissions from leaves on Oak logs in the laboratory 2016

Gary Battell and Tom Newman filming, and James Doonan stripping bark off a tree with unknown disease with weeping stem patches in Shropshire 2017



Carrie Brady using the confocal microscope to examine fine sections of Oak twigs inoculated with fluorescently-labelled B. goodwinii *2019*

Filling the liquid nitrogen cannister for omic sampling at Sotterley 2018

Viky Bueno-Gonzalez checking on her AOD bacterial samples grown without oxygen in the anaerobic chamber 2019

Jozsef Vuts installing a climbers platform to support air entrainment apparatus in the Forest of Dean 2018

Gary Battell recording scientific activity in Staverton 2016

Tree climbers sampling foliage at Bigwood 2016

Personal reflections on nine years of AOD research

James McDonald, Bangor University

For nine years now I have had the privilege of being part of this wonderful community of scientists and stakeholders that shares the common goal of safeguarding our native Oaks. However, as a scientist with no prior background in plant pathology, my involvement in AOD research began under unusual circumstances. As an undergraduate, I studied microbiology with a focus on medical microbiology and virology, and was fascinated by the complexity of human pathogens and their virulence mechanisms. Subsequently I planned to undertake a PhD in medical microbiology, but I became curious about an advertised PhD on the ecology of microorganisms that break down cellulosic biomass in radioactive waste landfill sites, and the opportunity to diversify into a project that explored the role of microorganisms in waste management and biomass decomposition led me to switch focus. I never would have predicted that I would end up working in the field of forest pathology, but the beauty of being a scientist is that knowledge and skills are transferable; you can indulge your natural curiosity for science, move into new research areas that often bring new perspectives.

In March 2010, I started a lectureship at Bangor University and was in the process of establishing my own research group. One evening I arrived home from work, and as my wife (who is a teacher) had a parents' evening and would be home late, I made the unusual decision to eat dinner in front of the TV. The BBC's 'One Show' came on, and a report on a mysterious disease affecting native Oak caught my attention. There was a scene in the field where Sandra Denman was describing the symptoms of AOD and the identification of several new bacterial species. This was my 'Eureka!' moment. The AOD situation perfectly combined my somewhat disparate interests in pathogens, plant biomass degradation, and nature, and I could see where my expertise in molecular analysis of microbial communities and biomass decomposition could be useful. The following day, I contacted Sandra Denman and subsequently, over several months, we developed some collaborative ideas to investigate the role of these bacteria in AOD.

In the last nine years we have come a long way, developing pioneering approaches to characterise the role of the

James McDonald in the Laboratory at Bangor University

James McDonald and Emma Ransom-Jones sampling AOD trees

microbiome (the collection of microorganisms and their genes in an environment) in healthy and diseased Oak trees, identifying causal agents of tissue necrosis, and beginning to reveal the virulence mechanisms that cause stem tissue necrosis in AOD (see other articles in this issue). Funding from Woodland Heritage, its sponsors, and Defra have been instrumental in making such progress. Bangor University has a rich history of excellence in forestry and has been one of the largest recipients of Woodland Heritage funding which has enabled retention of key members of the team, ensuring progress at important phases of the research programme, and facilitating our close collaboration with Sandra's group at Forest Research and the wider team. Across the wider AOD research consortium, this consistency of personnel over several years has meant that the group has a real family feel to it, and our collaborations have developed into friendships which make the whole project work so effectively. As the UK funding landscape is very competitive, collaborations tend to be more ephemeral and responsive to funding availability which is usually short term. Thus the fundamental role that Woodland Heritage and its supporters have played in driving forward a sustained AOD research programme is now paying real returns.

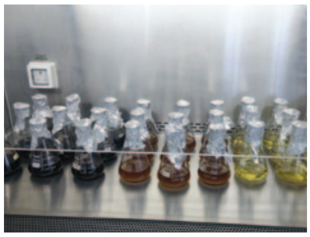
This journey has been a fantastic experience but is not yet complete. Peter Goodwin's tenacity and rallying call to hit these problems head-on continues to inspire, and collectively, I believe that the resulting research effort from the team is substantial and world-leading, and will leave a lasting legacy that will ensure that tree health can be safeguarded for future generations.

Challenging orthodoxy within plant pathology research

James Doonan, Post-Doctoral Researcher, Bangor University

In 2018 AOD research teams at FR and Bangor University, including myself and Professor James McDonald, published an article in the journal of the International Society for Microbial Ecology (ISME) proving, through empirical evidence, that AOD stem necrosis is caused by a community of bacteria [36]. This was a landmark study which applied traditional microbiology methods and modern molecular sequencing techniques to challenge the prevailing theory of Koch's postulates which requires the pathologist to prove that a disease can be recreated in a model system through the action of a single putative pathogen. Through this work we were able to prove that a community of micro-organisms dominated by *Brenneria goodwinii*, and to a lesser extend *Gibbsiella quercinecans*, can break down Oak tissue into component sugars.

James Doonan (Post-Doctoral Researcher and Bethany Pettifor (PhD student)



Shaking incubator with bacterial cultures

Subsequently, in a study published in the Microbiology Society journal, Microbial Genomics, we analysed bacterial DNA from necrotic AOD lesions including *B. goodwinii*, *G. quercinecans* and other infrequent isolates, including *Rahnella victoriana* and *Lonsdalea britannica* [47]. We sequenced the complete genomes of these bacteria and compared their DNA coding regions (coding regions are genes which are first transcribed and then translated into proteins) to a selection of bacteria. This selection included the bacterial plant pathogens *Pseudomonas syringae, Agrobacterium tumefaciens* and *Pectobacterium carotovorum*, and bacteria associated with healthy plants including commensal bacteria (harmless to the plant and may offer protection against invading pathogens) and growth promoters (bacteria which provide the plant with nutrients, for example through nitrification of nitrite to nitrate).

Our analysis revealed that bacteria consistently isolated from AOD lesions constitute a pathobiome (a bacterial community associated with disease) – providing additional evidence that necrosis is caused by multiple bacteria and not the action of a single pathogen. Furthermore, we found that *B. goodwinii* and *L. britannica* have many traits which are similar to those of characterised pathogens, primarily a type III secretion system and effector proteins (the type III secretion system is the primary virulence mechanism in seven of the top ten bacterial plant pathogens - it is a complex nanomachine that injects proteins from the bacteria to the plant) [41]. The prevalence of *B. goodwinii* in the lesion environment combined with DNA sequence information creates a body of evidence showing that *B. goodwinii* is likely to be the foremost contributor to AOD tissue necrosis. However, we know that *B. goodwinii* is able to cause disease only with additional help. This work identified, through DNA sequence analysis, that pathobiome members including *G. quercinecans* and *R. victoriana* contribute to necrosis as secondary/opportunistic pathogens leading to the proposal that they are generalists (i.e. they are able to adapt to varied environment conditions) and help *B. goodwinii* survive.

Collaborative studies between FR and Bangor University have been funded by Woodland Heritage and particularly the Rufford Foundation and the Monument Trust, via Woodland Heritage. This funding has allowed us to prove that AOD tissue necrosis is caused by bacteria and allowed us to challenge the prevailing orthodoxy that one pathogen equals one disease as stipulated by Koch's postulates. We have shown that Oak trees predisposed by environmental stress can fall victim to a weak bacterial pathogen – *B. goodwinii* and are easy prey for a co-operative bacterial pathobiome to eat away at our cultural heritage.

Bringing tree ring science to the study of Oak decline

Mary Gagen, Josie Duffy, Neil Matthews, Rebecca Pike, Giles Young

Our team is part of the Tree Ring Research Group at Swansea University. Most of us are climate scientists by training and study the rings in trees to explore past changes in weather and climate patterns. In 2012, with the support of Woodland Heritage, we were delighted to join Sandra Denman's group of scientists studying Oak decline. Tree ring records can provide useful insights into how decline has developed through time and allows us to study the differences in growth between healthy and declining trees, back through time. PhD geography students Josie Duffy, Neil Matthews and undergraduate Rebecca Pike have all, thanks to funding from Woodland Heritage, been able to carry out research projects in the Forest of Dean, at sites profoundly affected by Chronic Oak Decline (COD).

Their work has revealed that trees show the onset of decline in their annual growth rings long before tree crowns show symptoms, and that declining trees struggle progressively to produce trunk wood and therefore produce only very narrow rings indicating poor growth and vitality. The core image shown here (top right) reveals tiny tree rings, of just a few millimetres, in the years when COD was first noted (from 2010) at Chestnuts Wood in The Forest of Dean. Our students have also been able to develop the use of stable isotope analysis of tree ring samples to explore further how decline develops over longer timescales, revealing changes in how declining trees photosynthesise and produce carbohydrates, decades before the crown condition and root systems show symptoms. As a PhD supervisor and mentor to this fantastic team I can say for certain that their development as scientists has benefitted hugely from working as part of the wider decline team of the FR led consortium.

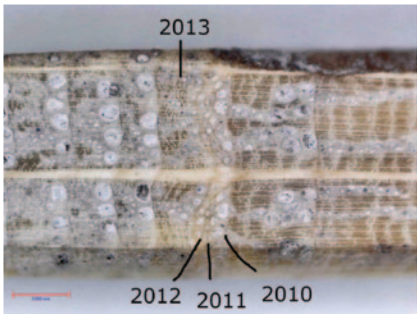
Thanks to the support of Woodland Heritage they have been able to test their ideas through field and lab work, and benefitted from the wider expertise of pathologists, foresters, genetics experts and those managing our vital Oak woodlands. They have gone on to publish their findings and present them at international conferences. Josie Duffy recently submitted her PhD, Neil Matthews will complete his next year and Rebecca Pike gained a first-class honours degree in geography and has gone on to study for her Masters.

Coring tool

Mary Gagen

Rebecca Pike taking a core

Neil Matthews



The annual growth rings, earlywood and latewood, in an English Oak tree (Quercus robur) *are shown. The very narrow years of growth in 2010, 2011 and 2012 at Chestnuts Wood are indicated*

Josie Duffy taking a core

Detection of AOD associated bacteria in Oak stem bleeds

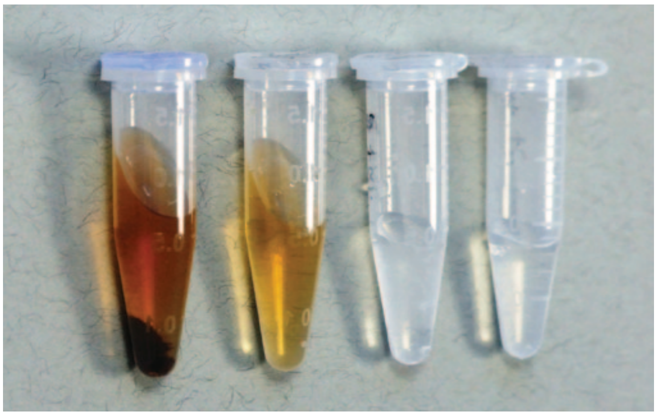
Bridget Crampton, PhD Plant Pathologist, Bangor University

Acute Oak Decline (AOD) is a syndrome affecting mature Oak trees, and is characterised by stem bleeds from vertical fissures on Oak trunks, irregular lesions in the inner bark beneath the bleed area, and often presence of larval galleries of the buprestid *Agrilus biguttatus* close to the lesions. Experiments have shown that four bacterial species, *Gibbsiella quercinecans*, *Brenneria goodwinii*, *Rahnella victoriana* and *Lonsdalea britannica*, are frequently associated with symptomatic Oak. Forest Research (FR) receive numerous bark samples from the public every year for AOD confirmation, and thus laborious culture methods to identify AOD associated bacteria are not feasible. FR has therefore developed a rapid screening assay to detect and quantify all four bacteria simultaneously. This multiplex quantitative polymerase chain reaction (qPCR) assay relies on the detection of gene fragments that are specific to each of the bacterial species in question.

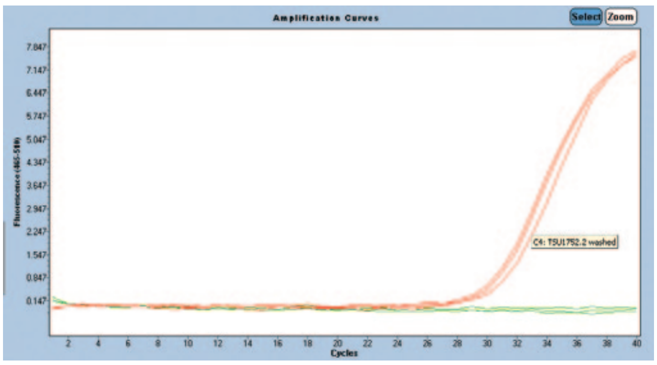
Bridget in the lab carrying out swab testing

Swabs for testing

Members of the public who suspect an Oak tree has AOD simply need to take a swab sample of the Oak stem bleed and send it to FR. We will elute bacteria from the swab and test for the presence of AOD associated bacteria using the qPCR assay. It costs approximately £6 plus staff time to process a swab sample and detect AOD bacteria, and up to 40 samples can be analysed in a day. We process a few hundred samples every year and FR currently runs this as a free service in the interests of assessing AOD distribution and spread across the United Kingdom and Europe.



Preparation of swab testing involves washing and centrifuging the exudates in the swab. From left to right: initial swab washing showing the progressive removal of tannins and other phenolic compounds that interfere with the testing chemistry.



Curve showing amplification of bacteria from swab samples

Oak health, the rhizosphere microbiome and soil chemistry: Is there a link between belowground and aboveground environments?

Diogo Pinho, Conceição Egas, Elena Vanguelova and Sandra Denman

In 2016 Forest Research and Biocant (Portugal) researchers joined forces to understand the health of declining Oaks. This fruitful collaboration started after the COST action FP1305 (funding network dedicated to scientific collaboration) granted Diogo Pinho, a PhD student from Portugal, a short-term scientific mission fellowship to visit FR at Alice Holt. In this work, we are particularly interested in the bacterial and fungal communities (microbiome) that live in the Oak rhizosphere soil. This soil is attached to the fine feeder roots of trees and is influenced by the interactions between tree root secretions and associated soil microorganisms.

Rhizosphere soil attached to Oak fine roots

The rhizosphere has specific chemistry and is rich in microbial diversity, abundance and activity. The rhizosphere microbiome helps trees by enhancing nutrient uptake and protects plants against abiotic and biotic stressors, and therefore it is expected that trees rely on these communities for their growth and health. As such, it is crucial to know which microorganisms are living in the rhizosphere and what their function and activity are, to ultimately improve the well-functioning of our iconic Oak trees.

We sampled the rhizosphere of healthy and AOD symptomatic mature Oak trees (over 200 years old) in three UK parklands to obtain insights into the links among tree health condition, microbiome, and the soil environment. For that we used molecular techniques, namely metagenomics approaches, to characterise bacteria and fungi, even those that are not suitable for culturing, and we also analysed the physicochemical properties of the sampled soils.

The results showed clear links among tree health condition, rhizosphere microbiome and soil chemistry. Across the three sites, the Oaks at Richmond Park (London) had the most numerous and severe stem bleeds and had suffered a long time with earliest records dating back to the 1980s (FR records). Both asymptomatic and AOD trees were characterized by very acidic soils around their roots and their rhizosphere microbiome was distinct compared to the other two sites. By comparison only the AOD trees at Eastnor Castle Deer Park (Ledbury) and Hatchlands Park (Guildford) had their rhizosphere acidified and showed differences in the microbiome composition compared to healthy trees. Notably, microorganisms that live and feed on dead organic matter were more abundant in Richmond and bacteria and fungi known to be beneficial to trees were more abundant in the rhizosphere at Eastnor and Hatchlands.

It is well known that in times of stress, trees undergo induced changes in their root exudations and rhizodeposition takes place, changing the existing rhizosphere properties. Acidification of the rhizosphere is one such process that trees use in an effort to counter poor soil environments and mobilize nutrients for uptake to sustain growth and defence. At Richmond the soil is naturally acidic so further research is required to understand the interactions between soil chemisrty and microbial communities. At the other two sites however, it was only the rhizosphere soils around the AOD trees that were acidified, suggesting tree-microbial-soil interactions to benefit nutrient uptake by the trees. These results raise the exciting hypothesis that soil condition and belowground rhizosphere communities can potentially protect Oaks against AOD, a decline-disease with aboveground symptoms. This is the very first step and much more work needs to be done to better understand the role of soil condition and rhizosphere communities on AOD establishment. In long term, this knowledge may be included as part of a management plan in protecting our British iconic Oak trees against AOD.

Diogo working beside Oak tree

Linking environmental factors and belowground biodiversity with Oak health

Elena Vanguelova, Sue Benham, Frank Ashwood, Forest Research

To understand the reasons for Oak Decline it is vital to investigate the impact of biotic agents and their interactions, but it is critical to consider first the whole system beginning with linkages to environmental factors at the landscape level as well as at the individual tree scale. The subject area that is able to do this is biogeochemistry. I am a biogeochemist based at FR, Alice Holt, and a few years back Sandra Denman invited me to work with her and the AOD team on a Defra funded project called FPPH Oak Resilience, which finished this March. We have been on an amazing journey together and now have evidence of a number of clear predisposition drivers of Oak Declines (both AOD and the more traditional COD – Chronic Oak Decline).

In AOD by using data collected by Nathan Brown and FR's citizen science reports for more than 500 locations, Nathan and I mapped and spatially modelled AOD occurrence against soil type, climatic factors and chemical deposition (nitrogen (N), sulphur (S) and base cations – for example calcium (Ca), magnesium (Mg), sodium (Na) and potassium (K)). The results showed that AOD in England and Wales is significantly influenced by rainfall, air temperature, and elevation, as well as nitrogen, sulphur and base cation deposition. This knowledge underpins risk mapping and will help develop best practice management advice.

The spatial analysis at the landscape level also highlighted the potential effects of soil types and soil moisture, however these needed to be investigated at smaller scales, e.g. at site and tree level. The spatial study also re-emphasised the importance of predisposition factors in understanding the onset of Oak Declines at smaller scales. In the FPPH project, a site/tree specific investigation on ten sites that Nathan and Sandra have been monitoring for ten years and acquired pathological histories and other important information, the links between soil biogeochemical indicators, soil fungal and microbial diversity, and the relationships of these with Oak health and nutrient status are being investigated.

Our first results are showing that soil type and its associated physical, chemical and biological properties

Eastnor Oaks

Joe McMinn, Forest Research Technical Support Unit with Elena Vanguelova, Biogeochemist

regulating the main soil functions play a significant role in Oak nutritional and health status. One emerging result suggests that nutrient imbalances in diseased vs healthy trees which have been discovered on a COD site, has strong correlations with the phenotypic models assessing tree health condition developed by Jasen Finch. Other preliminary results suggest strong links between belowground traits such as tree feeder root distribution, morphology and biochemistry, soil microbial and fungal communities, and tree health. Once these relationships are clearly established further research to obtain evidence of proof of cause and effect, will be required so that appropriate management for protective and ameliorative actions can be developed and recommendations made.

Furthermore, as part of the FPPH project, we hypothesised that parkland Oaks could be exposed to more unfavourable soil environments compared to Oaks in woodland settings. Preliminary results are showing that soil under Oaks in parkland settings commonly have higher compaction and thus less water and oxygen provision, but also higher acidity, surface soil erosion and lower organic matter content compared to soil under Oaks in woodland settings. Thus we suggest that the response of Oak to abiotic and biotic predisposition factors in different woodland settings should form the scope of follow-on research to determine the vulnerability and resilience of Oaks in the wider landscape in Britain.

Without the multidisciplinary collaborative work that Woodland Heritage and Defra make possible, these insights may not have been gained and so many thanks are given to these organisations for the opportunity to be part of such a dedicated, enthusiastic team and to investigate these core underpinning relationships that influence Oak health.

Oak health and mycorrhizal fungi

Laura M. Suz (RBG Kew) and Martin I. Bidartondo (Imperial College)

It is not only aboveground where Oaks harbour a high diversity of organisms, but also belowground in their roots and many of these are highly beneficial organisms. More than 90% of the feeder roots of Oaks are covered by fungal tissue forming ectomycorrhizas, or "outer fungus-roots". In this ancient and intimate symbiosis, fungal filaments fully sheath the fine roots and grow in between their outer cells. In fact, nearly all nutrients, such as nitrogen and phosphorus, enter Oak via this fungal pathway, and Oak interactions with soil, water and microbes are also mediated by fungal tissue. In return, trees allocate up to 20% of their carbohydrates to ectomycorrhizal (ECM) fungi that use them to fuel mining, scavenging and transporting resources in the soil. Therefore, to understand how Oaks interact with soils regarding nutrient and water uptake, and protection from attackers, we need to understand their mycorrhizas.

Laura M. Suz (PI, RBG Kew)

Martin I. Bidartondo (PI, ICL and RBG Kew)

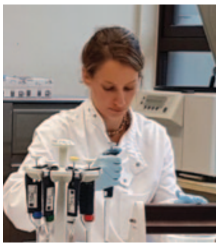
Morgan Peas (MRes student, ICL) and Alexander Borg (Research assistant, RBG Kew)

Oak tree roots harbour a diversity of fungal species forming ectomycorrhizas in their roots, with different species showing a great variety of morphologies and functional traits that maximise nutrient uptake from soils in different ways. Note the fine hair-like mycelial threads of the fungi entangled in, and emanating from the Oak feeder roots.

In October 2018 we joined the FPPH project, aiming to contribute to the combined efforts to understand and mitigate Oak decline in the UK. We are a small team based at Kew that has worked together for 15 years on the ecology of mycorrhizas with a particular interest in the factors that affect their diversity and distribution. We have found evidence, for instance, that ECM fungi of Oak are strongly affected by biotic and abiotic factors, forest management practices, and environmental change. Moreover, we identified ECM fungi of Oak that can be used as indicators of forest condition, and generated the first baseline ECM diversity data for Oak forests across Europe that can be used to assess future forest changes, a must for robust scientific studies in our rapidly-changing world.

Changes in fungal richness and abundance affecting certain ECM species with different capabilities with regard to storing carbon in soil, and taking up and transporting mineral nutrients, can have important consequences for tree nutrition and forest ecosystem stability and functioning, with potential consequences for Oak health and susceptibility to pests and diseases. It remains to be tested whether changes in ECM communities are causal factors of early stages of Oak decline or not, and how ECM fungi interact with other factors triggering Oak decline. We still know too little about the link between functional diversity changes in ECM communities and Oak health, so this project represents a great opportunity to start filling a basic knowledge gap.

Funded by Defra and The Forestry Commission, we have sampled ectomycorrhizal roots of healthy and declining Oaks (AOD, COD) in nine parklands and woodlands in England. In the laboratory, over 2,000 ectomycorrhizas were selected for identification using DNA-based methods. We also recorded their morphological traits to get information about how these fungi explore the soil and to infer the carbon and nitrogen dynamics in these plant-fungal interactions. Our main aim is to explore the links among ectomycorrhizal diversity, biotic and abiotic stressors, and oak health. We hypothesise that there are differences between the ECM communities in roots of healthy and declining trees, and that oak health decline is driven by a drop in the functional diversity of these essential fungi, and that changes in biotic and abiotic factors are linked to changes in ECM communities. Overall, we expect to generate diversity data on ECM fungi associated with healthy and declining trees, and to generate guidelines for improving oak health through innovative targeted management for mycorrhizal diversity.

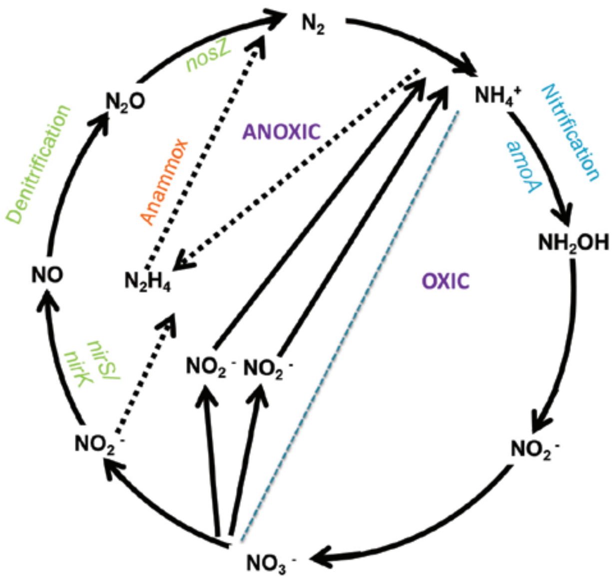


The microbiology of a healthy Oak soil – the nitrogen balancing act

Kelly Scarlett, Sandra Denman and Corinne Whitby

Nitrogen is an essential building block for plant growth. Microorganisms play a critical role in making nitrogen (N) available to plants to take up by cycling it, particularly in terrestrial environments such as forest soils. In the N-cycle, nitrification involves the conversion of ammonium (NH_4^+) to nitrate (NO_3^-) via nitrite (NO_2^-) (as shown below) and this is carried out by ammonia oxidising bacteria (AOB) and archaea (AOA), which are single celled organisms like bacteria. Another group of microbes known as denitrifying bacteria use the excess NO_3 from nitrification processes and perform a stepwise reduction of NO_3, to nitric oxide (NO), nitrous oxide (N_2O) and dinitrogen gas (N_2). This process is called denitrification, and it takes place to create a dynamic balance between availability and excess in plant growth systems.

In forest ecosystems, intensive farming methods in surrounding land have caused environmental pollution to increase over the last 50 years due to an increase in nitrogenous fertilisers, pesticides and organic waste which, while initially causing excess N input, have led to an increased loss of N from the system via leaching and denitrification.



The nitrogen cycle

In the UK and Europe, Oak ecosystems are experiencing the effects of man-induced fluctuations of N and acidifying compounds. It is hypothesised that these fluctuations are destabilising tree available nutrients, and reducing stress tolerance of Oak trees. Therefore a functional balance in microbial communities must be achieved, harnessing the much needed N with those that counteract excesses, which are crucial to maintaining 'soil health'.

In order to improve managing forest soil systems as a whole, we need to better understand the close link between the various microbial communities that are actively involved in these N transformation processes, and how changes in the environmental / soil parameters might disturb this fine balance. An essential part of this understanding is knowledge about the effects of N on Oak decline and the role of the soil microbiome in mitigating these effects, which are currently unknown.

Kelly Scarlett collecting field samples

Kelly and Corinne joined the FR AOD consortium led by Sandra Denman as part of the Defra FPPH funded project. The aim of this particular study is to characterise and assess functional balance of the soil microbial communities involved in nitrification and denitrification in relation to Oak tree health. Outputs from this project will enable more informed soil and forest management practices, leading to better process control and general management of forest resources.

Oak genomics and AOD

Richard Buggs, Kew, and Gabriele Nocchi, PhD student, Kew and Queen Mary University of London

Are there DNA-based differences among Oak trees that affect their susceptibility to AOD? Scientists at Kew Gardens have started work on this question in the hope that they may discover inherent resistance to AOD.

Samples from Oak trees from five sites were collected by the FR technical support team (FR TSU) in autumn 2017. The sites were: Attingham Park, Chestnuts Wood in the Forest of Dean, Hatchlands Park, Langdale Wood and Sheen Wood in Richmond Park. These sites have been monitored by FR and Nathan Brown from Rothamsted Research for some years, and have been the sites for many studies on AOD undertaken by the FR led AOD consortium. Phenotypical characteristics including AOD status, crown condition, age and the diameter of these trees were accurately recorded and compiled by FR and Rothamsted Research. To find out whether there are genetic differences amongst the individual trees and tree populations that could confer susceptibility or resistance to AOD, DNA was extracted from these trees by Prof. Richard Buggs' group at Kew. The whole genome sequences (all the DNA) of over 400 trees were sequenced in Hong Kong. This generated over ten terabytes of data. This is being analysed by Gabriele Nocchi, a PhD student at Kew and Queen Mary University of London. He has mapped all the sequence data from the 400 trees to a high quality reference genome assembled by scientists at INRA, France. The Oak genome is 750 million DNA letters long, and Gabriele is finding the millions of these letters that vary among English Oak trees.

Gabriele's long-term aim is to assess whether differences between the genomes of Oak trees cause differences in their susceptibility to AOD symptoms. Thus we will find out whether or not it will be possible to breed Oak trees with resistance to AOD.

This work is funded by Defra, by a grant to Kew under the Action Oak initiative.

ActionOak
PROTECTING OUR OAK TREES

Gabriele Nocchi, a PhD student in Richard Buggs' Lab

Richard Buggs inspecting an Ash tree

Farewells and highlights, Promotions and honours

Farewells

Sarah Plummer joined the AOD research programme as a gap student in November 2012 and from 2013 - 2015 she was employed by Bangor University but based at Alice Holt as a research assistant to Sandra Denman. As part of the AOD team she was funded by Woodland Heritage. In 2015 Sarah obtained a permanent senior pathology technician post at Alice Holt. In January Sarah left this position to join the RBG team at Wisley. Sarah has contributed a great deal to the AOD research programme. She showed much skill in her log test work and will be remembered particularly for the painstaking work she did transferring newly hatched *Agrilus* larvae using a single bristled eye makeup brush, into logs in inoculation tests. Sarah also processed many swab tests using quantitative polymerase chain reactions (Real Time PCR) and getting those all-important results to anxious landowners and managers. We thank Sarah for her hard work and wish her every success in her new job. She will be missed by her friends at Alice Holt, and by the British Oaks that she served.

New arrivals

Katy Reed gave birth to Miles Anthony in February 2018 and Nathan is father to daughter Edith Philippa Freda born in July 2018 – Congratulations to both families, may you all be blessed with happiness and good health.

New scientists joining the team

Dr Bridget Crampton has joined Sandra Denman's lab team on a three year Defra funded contract. Bridget has come to us from the Forestry and Agricultural Biotechnology Institute in Pretoria South Africa, and is working on a project aimed at discovering the cause of a disease in Oak at a site in Norfolk.

Dr Anbu Kajamuhan joined Sandra Denman's lab team where she is giving laboratory assistance.

Mark Ferryman who is a research assistant at Alice Holt has been helping our team with GPS Trimble mapping, data acquisition in the field, the laboratory rearing of *Agrilus* and media preparation and general laboratory duties in our pathology laboratory. We are delighted with Mark's skilled and versatile help.

Bethany Pettifor has joined the group on a Defra funded PhD. She has begun working on *Gymnopus* buttress root rot on Oak, (in old currency called *Collybia* buttress root rot), under the leadership of Sandra Denman and James McDonald.

Welcome to the AOD family, we look forward to working with you and doing great science together.

Promotions

Dr Daegan Inward (FR) has been promoted to a Senior Entomologist, and Dr James McDonald received Professorship at Bangor University – Congratulations to you both, very well deserved and we are very proud of you.

Awards

The Action Oak Initiative had a stand at Chelsea, led by Dr Paul Beales (APHA), which was awarded a Gold Medal. Daegan, Katy, Clive Muller, Bridget and Sandra prepared an AOD display, and Sandra contributed to manning the stand.

Recognition

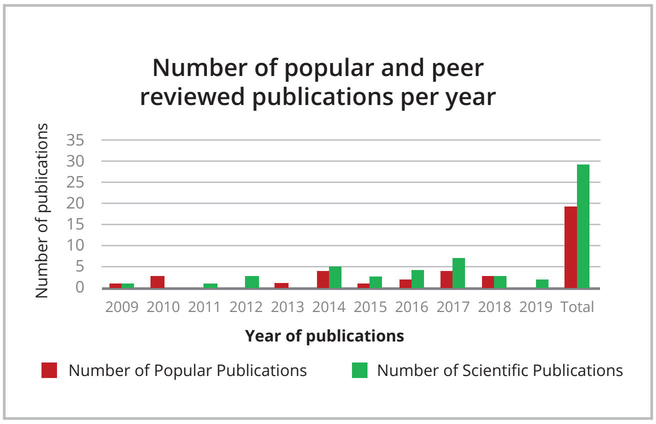
Dr Sandra Denman was invited to give 'The Ethel Doidge Memorial Lecture' at the South African Society of Plant Pathology annual Congress and also to give a lecture at the International Oak Society Meeting in Davis, California.

Honours

Dr Joan Webber, who was involved in the AOD story at its inception, and has kept a parallel interest in its development, was awarded an OBE for her contribution to Forest Pathology. Well done Joan, richly deserved recognition!

Publication Record

We are proud of our publication record, 48 articles in 10 years.



Sarah Plummer on field work in the Forest of Dean

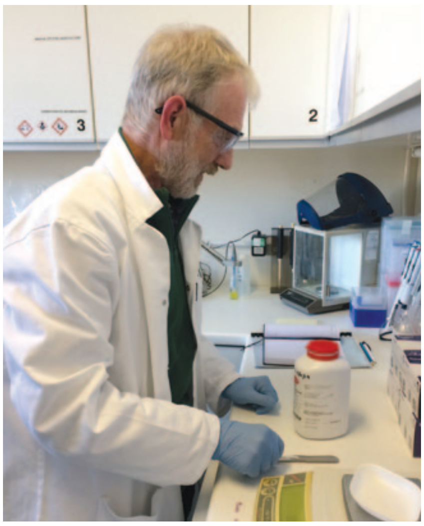
Anbu Kajamuhan in FR Lab

Bridget Crampton and Anbu Kajamuhan at Sotterley, Suffolk

Daegan Inward, Ed Eaton, Katy Reed baking cores at Attingham to study dates of Agrilus colonisation using dendrochronology

Kelly Scarlett and Sarah Plummer working on log trials

Professor James McDonald



Mark Ferryman working in FR pathology lab

Sandra Denman manning the Chelsea stand

Joan Webber who recently received an OBE for her contribution to tree health in the UK

A valley Oak (Quercus lobata) in the Consumnes Preserve, California.
These trees belong in the white Oak group, often tower to 35m high, with a DBH of 4m, the bole beginning at 5-10m above soil level, and the trees have long spreading branches giving it a very large width. The spread of the crown of this tree was paced to approximately 40m. These trees are threatened due to loss of habitat

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Following similar publications in 2015 and 2017, this is the third standalone Acute Oak Decline (AOD) Newsletter, which coincides with two notable landmarks for Woodland Heritage: a decade completed of raising funds for research into AOD and 25 years since its incorporation in 1994.

Woodland Heritage is a small charity, so for its Co-Founder, the late Peter Goodwin, to have recognised the problem and to have inspired others to give so much for AOD research (£2.5m to-date and still rising), was a remarkable achievement and a true legacy for the benefit of the environment and society in the UK, as both would be lost without our iconic Oak.

The list of donors over the last decade is extensive and to all we express our profound gratitude, but particular thanks and credit are due to The Monument Trust, The J. Paul Getty Jr Trust, The Gatsby Charitable Foundation, The Rothschild Foundation, The Rufford Foundation and Lord Sainsbury of Turville.

Woodland Heritage is proud to be associated with the research described in this Newsletter and to have had the chance to work with the many scientists who have contributed to what is a rapidly accumulating wealth of knowledge on the likely causes of, and potential ways to mitigate against, AOD.

Before Peter's death in March 2017, his approach to raise funds from private donors, to enable a charity to contribute towards research often pursued by public bodies, inspired a way of working that led to the creation of 'Action Oak'.

Launched officially in 2018, this unique initiative aims to look at all threats facing the Oak, including from AOD, and to seek support to tackle them all in a holistic and joined-up way. The members of Action Oak are Woodland Heritage, Woodland Trust, the Duchy of Cornwall, Royal Botanic Gardens Kew, the National Trust, Forestry Commission, Forest Research, Defra, the Northern Ireland Forestry Service and the Governments of both Scotland and Wales.

Action Oak will ensure that every bit of funding is spent to the greatest advantage and you can help to raise the vital funds required by making a donation at woodlandheritage.org/acute-oak-decline

Our last word is devoted with great pleasure to the scientist who has been at the heart of AOD research for the last decade and continues in that role today, seemingly with undimmed energy and determination. Sandra Denman is strong in her praise of every member of the research team in this Newsletter, but she is perhaps understandably modest when it comes to recognising her own skill both in her own research and, possibly even more important, her leadership skills shown in the way in which she has formed, motivated and directed the very effective team of AOD scientists. We at Woodland Heritage, and now in Action Oak, say: "Thank you, Sandra, and well done!"

Lewis Scott and Guy Corbett-Marshall
Woodland Heritage

WOODLAND HERITAGE
Celebrating 25 years

Woodland Heritage is a registered charity number 1041611