



# UK Oak biogeochemistry – now and in the future

Elena Vanguelova, Forest Research


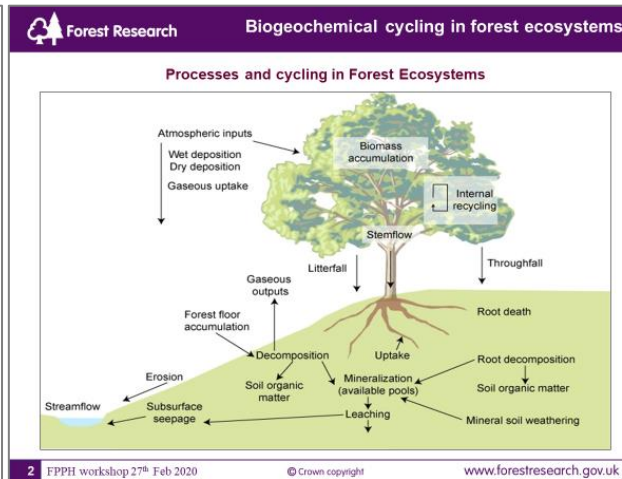
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## The Oak biogeochemistry – now and in the future

**Elena Vanguelova**

Sue Benham, Nathan Brown, Frank Ashwood, Jack Forster, Sandra Denman

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WP1: Biogeochemistry


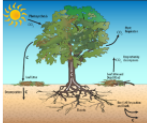

**Question:**  
Are there identifiable predisposition drivers in oak declines at UK AOD and COD sites

**Hypotheses:**

1. There are identifiable pre-disposition drivers associated with AOD and COD
2. Predisposition factors vary at different scales (national, site, tree scales) and tree condition (AOD/COD)
3. Altered and impaired feeder root morphology and chemistry are linked with predisposition factors
4. Altered and impaired tree nutrient uptake is linked with soil conditions and poor root development
5. Linkages occur between soil predisposition factors, tree root and foliage parameters

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### Essential nutrients for trees

macronutrients	micronutrients
Nitrogen (N)	Iron (Fe)
Phosphorus (P)	Manganese (Mn)
Potassium (K)	Zinc (Zn)
Calcium (Ca)	Copper (Cu)
Magnesium (Mg)	Boron (B)
Sulphur (S)	Chlorine (Cl)
	Molybdenum (Mo)

N, P and K make 75% of mineral nutrients in plants  
All 13 nutrients are required for tree health and growth

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### Hypothesis testing



Have we addressed our Hypotheses?

1. Identifiable pre-disposition drivers associated with AOD and COD ✓
  - Cause and effect studies required related with potential soil drivers (N, C, P, pH, Ca, Mg, K)
2. Predisposition factors vary at different scales (national, site, tree scales) and tree condition (AOD/COD) ✓
  - National scale - warmer, drier areas at lower elevations with high N and low S levels. Drought effects need testing under controlled conditions.
  - Between sites - calcium uptake inhibitions and changes in carbon and nitrogen cycling
  - Site specific scale – two scenarios emerging related to soil acidity and nitrogen and phosphorous deficiency
3. Altered and impaired feeder root morphology and chemistry are linked with predisposition factors ✓
  - Lack of feeder roots/ altered root morphology interfere with absorption and uptake of nutrients
4. Linkages occur between soil predisposition factors, root and foliage parameters ✓
  - Particularly clear in N and P content
5. Impaired tree nutrient uptake is linked with soil conditions and poor root development ✓
  - Lower tree nutrient uptake of N, P, Ca, Mg and K is linked with poorer soils and root development

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### Future research and needs

- To carry out controlled trials to further test nutrient and drought effects
- To expand monitoring to a greater numbers of trees so that the more generalised patterns can emerge
- Linking biogeochemistry data with fungal community data, rhizosphere microbiology, etc.
- To integrate aggregated data from FPPH to understand the prevailing factors, relationships and likely responses.
- Management trials (e.g. Chestnut to start with), but others can be useful such as in parklands.
- Cause and effects to be studied in controlled and field environment
- Expanding Oak trees health and biogeochemistry research into different settings, e.g. farmland, hedges, parklands, urban environment, veteran trees.

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**Elena Vanguelova<sup>1</sup>, Nathan Brown<sup>2</sup>, Sue Benham<sup>1</sup>, Frank Ashwood<sup>1</sup>, Jack Forster<sup>1</sup>, Sandra Denman<sup>1</sup>**

<sup>1</sup> Forest Research

<sup>2</sup> Woodland Heritage

Our research set out to identify predisposition drivers underlying acute and chronic oak declines in the UK. We analysed trends at the national scale (using over 500 monitoring sites where AOD was either absent or present) and local scale through detailed data acquired at 10 sites that had long-term monitoring information available. At each of these 10 sites specific soil, root and tree nutrient status were measured to compare declining and healthy oaks.

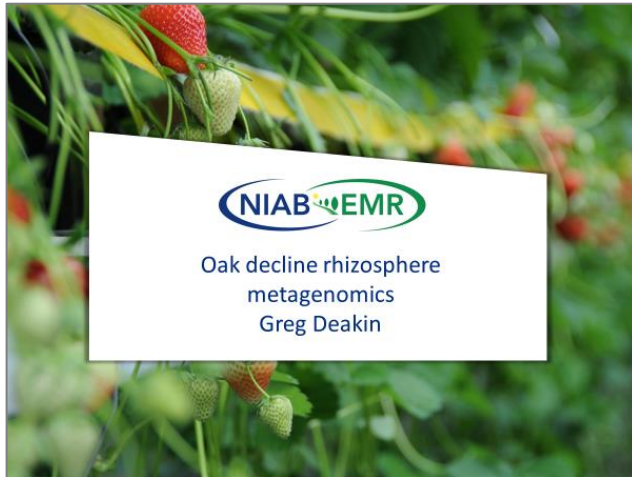
Initial analyses show that predisposition factors vary at the different scales (national, site and tree) and there are significant correlations with tree condition (AOD/COD). At the national scale, significant predisposition factors for AOD occurrence are climate, with warmer and drier areas, and atmospheric deposition with higher nitrogen and lower sulphur levels, being correlated with the occurrence of AOD. At smaller scales so far, we have observed soil nutrient and physical characteristics, including water holding capacity and soil sensitivity to drought to be prevailing factors. Potentially significant soil drivers include higher soil acidity, lower levels of plant-available nitrogen, phosphorous and calcium. Poor tree feeder root development and altered and impaired tree nutrient uptake are also linked to these soil conditions. Inhibition of calcium uptake and changes in carbon and nitrogen cycling in AOD and COD trees compared to healthy trees has also been detected. More in depth analyses are required to get clearer indications and identification of other significant interactions. The next steps in our analyses are to determine significant links between the biogeochemistry data, soil and root fungal community profiles and whether rhizosphere microbiology is significantly correlated to tree responses and the wider environment.

Future research will test the cause and effect relationships associated with oak decline, by manipulating the availability of specific nutrients to excess or deficiency levels, as well as artificially causing drought conditions. Additionally, in the wider UK context monitoring of oak health should be resumed from the 1980-2005 forest condition survey and data collection will be expanded so that the condition of UK oak can be modelled based on the tools we are currently developing. However, a broader range of site types to be monitored will be necessary to understand oak decline in different settings, e.g. farmland, hedgerows, parklands, urban environments and veteran trees where different factors may impact oak health. Management trials (in both forests and parklands) are needed to underpin management guidelines for oak resilience in future climate and other disturbance pressures.

Based on the results to date and future research we will be drawing more detailed guidelines highlighting significant predisposition factors and best practice to aid oak resilience, covering succession planting and more detailed site-specific risk assessments.

# Oak decline rhizosphere metagenomics

Greg Deakin, East Malling Research



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## Shotgun metagenomics

- At AOD sites: specific protein domains with increased abundance in AOD trees included a diverse range of regulatory process, e.g. protein modification and negative regulation of cellular proteins, and cytoskeleton.
- A number of protein domains of decreased abundance in AOD trees are related to interactions between organisms including immune response, response to bacteria, response to antibiotic and modification by symbiont of host morphology.
- There is some similarity in the proteins with increased or decreased abundance in AOD or COD. This is particularly true for the Langdale site for those bins with decreased abundance in AOD or COD trees

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## Metagenomics analyses

- Is there a difference in microbial diversity between healthy and diseased rhizosphere?
  - Amplicon sequencing (and metagenomics)
- Are there differences in specific microbes?
  - Amplicon sequencing (and metagenomics)
- Do the microbiomes (the collection of all microbes in a particular environment – the rhizosphere here) differ in their gene content?
  - Metagenomics
- Do the microbiomes differ in their protein content?
  - Metatranscriptomics (indirect method)

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## Amplicon sequencing

- There were no overall differences in rhizosphere microbial communities between healthy and symptomatic trees
- There were significant differences in the relative abundance of specific OTUs between samples from healthy and symptomatic trees.
- However, the exact differences vary greatly between sites, with very few common features. But, a possible reduction of beneficial microorganisms at AOD/COD site

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## Metatranscriptomics

- Numerous protein domains differed between diseased and healthy samples - but without clear interpretable patterns.
- No similarities between site in enriched GO terms and associated protein domains in COD. This indicates that the underlying microbial processes are different between the two sites (Attingham and Langdale).
- In contrast to the metagenomics data, there were no similarities between the AOD and COD results at the Langdale site.

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## Overall conclusions

- AOD or COD is not attributable to specific rhizosphere pathogens
- Tree health at **Speculation, Bigwood and Chestnuts** is unlikely to be directly associated with soil microbes.
- The lack of beneficial interactions among certain rhizosphere microbial groups may contribute to and/or outcome of the tree health at **the other four sites**.
- *Armillaria* infection may also be associated with tree health at Great Monks

# Oak feeder root and rhizosphere fungi, identity and pathogenic potential / Soil Baiting Tests

Sandra Denman, Forest Research



## Preliminary Results of Feeder Root Isolations and Soil Baiting Tests

Sandra Denman, Anbu Kajamohan, Bridget Crampton, Sarah Plummer, Kelly Scarlet

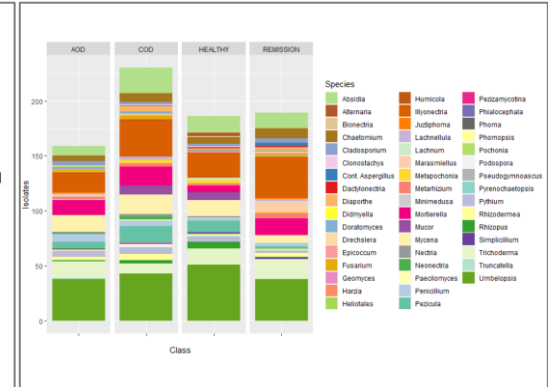
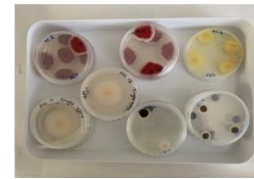
FPPH Workshop Alton  
February 2020

**Aim:** To isolate and identify fungi and oomycetes from oak feeder roots of AOD symptomatic and non-symptomatic trees and to determine whether they play a role in feeder root rot and function.  
Total number of isolates in hand 1192, about 20% complete

Isolates grouped based on gross morphology (often indicative of genus, occasionally more than one genus look alike) (>50 genera have been isolated)

Representative isolates within each group (genus) sequenced – uncovered different species – potentially some new species discovered

Within genera, based on initial ITS results, 2 or 3 representative isolates per species were single-spored to ensure pure colonies – multigenic sequencing, phylogeny and identification



## Soil Baiting Tests

### Aims

To detect Oomycetes in soils

- *Phytophthora*
- *Pythium*
- *Phytophthium*

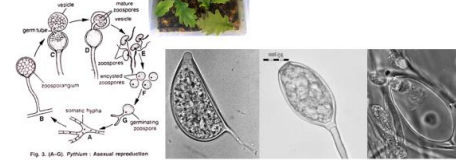
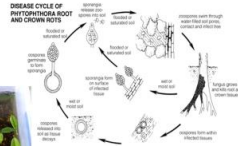


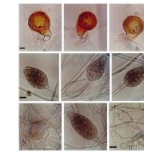
Fig. 3. (A-C) *Pythium* - Asexual reproduction



## Results of soil baits

- *Phytophthora europaea* (Bigwood)
- *Phytophthora cambivora* (Langdale)
- *Phytophthora gonapodyides* (Great Monks)
- *Phytophthora megasperma* (Attingham)
- *Phytophthora undulata* (Great Monks)
- *Phytophthora quercina* (Attingham, Langdale)

- *Phytophthium citrinum* (Winding Wood, Langdale)



Pythium species not yet recorded on oak in UK

In Europe *P. anandrum* and *P. intermedium* are considered feeder root pathogens on oak

<i>Pythium alternatum</i>
<i>Pythium anandrum</i> * (Turkey, Poland)
<i>Pythium aquatile</i>
<i>Pythium atratheridium</i>
<i>Pythium barbulae</i>
<i>Pythium catenulatum</i>
<i>Pythium coloratum</i>
<i>Pythium dissimile</i>
<i>Pythium dissotocum</i>
<i>Pythium glomeratum</i>
<i>Pythium glomeratum</i>
<i>Pythium heterothallicum</i>
<i>Pythium intermedium</i> * (Poland)
<i>Pythium mamillatum</i>
<i>Pythium rostratifingens</i>
<i>Pythium senticosum</i>
<i>Pythium spiculum</i> * (Spain)
<i>Pythium torulosum</i>
<i>Pythium ultimum</i>
<i>Pythium vanderpoolii</i>

## The Future

Complete initial sequencing and finalise genera and representative isolates

Get confirmed species identifications

Finalise data analyses to determine whether any species stands out either as a beneficial fungus or a detrimental pathogen

Do controlled tests to prove role



## Fungi associated with oak feeder roots in the UK

Sandra Denman, Anparassy Kajamuhan, Kelly Scarlet, Corinne Whitby\* and Bridget Crampton

Forest Research, \* University of Essex

Over the past decade we have undertaken intensive monitoring and integrated studies of oak trees on eight sites covering an east-west gradient in southern and central England where acute oak decline (AOD) occurs and thus have accumulated a wealth of information about the oak trees there. Studies have included the spatial epidemiology of the disease and predisposing factors where biogeochemistry was a central feature revealing at the national scale that AOD was more likely to occur in warm dry areas with high dry nitrogen and low dry sulphur levels. To take this study further soils, roots and foliage were analysed to determine whether there were linkages between these factors and tree health condition. Here, we report on the fungi associated with the feeder roots sampled during the wider biogeochemical study and on the presence of oomycete pathogens in the soils. We hypothesised that there would be differences in the feeder root fungal communities on healthy vs diseased oak. Four AOD sites were used Attingham, Langdale, Great Monks Wood, Winding Wood. Feeder roots were sampled at 4 cardinal points around each of 10 non-symptomatic and 10 symptomatic trees per site, and 10 root pieces were surface sterilised and plated onto a range of culture media. More than 1000 cultures were obtained but to date only around 25% of those have been identified. Results are thus preliminary. At least 50 different genera are represented among the 1192 isolates obtained. The most frequently genera of fungi occurring are apparently common saprophytes including: *Absidia*, *Alternaria*, *Mortierella*, *Mycena*, *Trichoderma* and *Umbelopsis*. Amongst commonly occurring pathogenic fungi *Illyonectria* was dominant and *Pezizula* was important, but both these genera occurred on symptomatic and non-symptomatic trees. These genera appeared to be universal amongst the sites and there did not appear to be differences between tree health condition. Further analysis may change the present picture.

# Ten years of bacterial taxonomy from oak – the path forward

Carrie Brady, University of the West of England

## Ten years of bacterial taxonomy from oak – the path forward

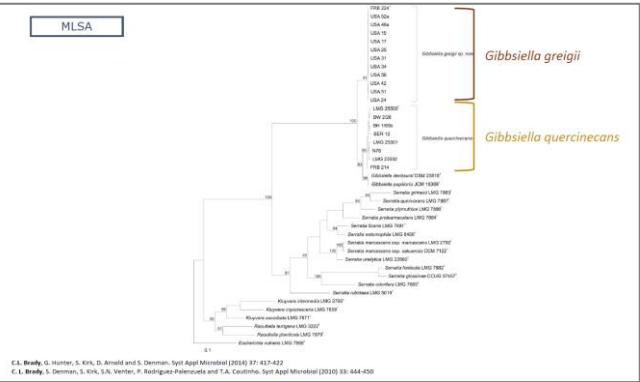
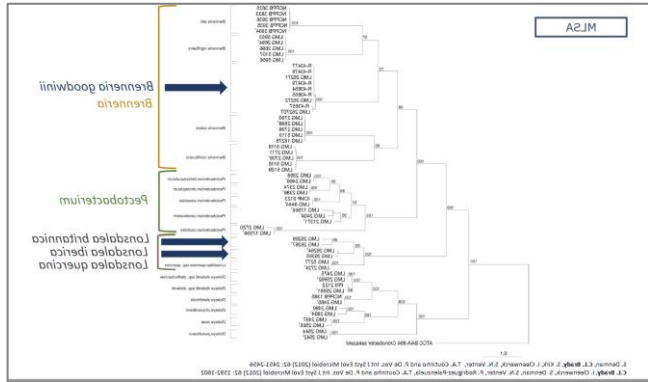
**Carrie Brady**  
**Sandra Denman**  
**Dawn Arnold**

### Acute Oak Decline bacteria

- 2008 → first bacterial isolations from symptomatic oak
- thought *Brenneria quercina* was causal agent
  - bark canker and drippy nut of oak
- one disease = one pathogen
- *gyrB* gene sequences → ‘*Serratia*’ species → no links to tree disease

### Bacterial taxonomy

- description, identification and classification of bacteria
  - assign isolates to correct species
- Linnaean taxonomy
  - lowest taxonomic rank for bacteria is subspecies
  - binomial nomenclature
  - Genus species eg. *Escherichia coli* (*E. coli*)
- historically grouped according to phenotype or disease caused



### 10 years of AOD taxonomy

- we have formally described:
  - 2 novel genera
  - 14 novel species
  - 5 novel subspecies
- studying oak microbiome has filled in gaps in bacterial classification
- contributed to catalogue of species

**Host-bacteria-insect interactions drive emergent virulence in AOD**  
James Doonan, University of Copenhagen

**Host-bacteria-insect interactions drive emergent virulence in AOD**

James Doonan<sup>1</sup>, Sandra Denman<sup>2</sup>, James McDonald<sup>1</sup>

Bangor University<sup>1</sup>, Forest Research<sup>2</sup>

Woodland Heritage and Forest Research funded project



03/03/2020

**Two bacteria consistently isolated from affected oak lesions**

- *Brenneria goodwinii*
- *Gibbsiella quercinecans*



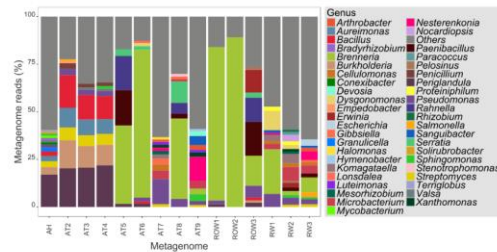
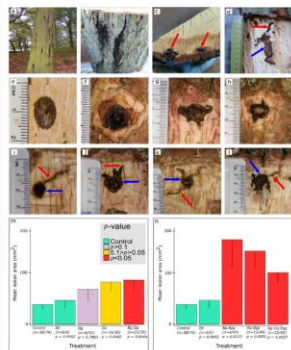
**Other bacteria frequently isolated**

- *Rahnella* spp.
- *Lonsdalea britannica*
- *Brenneria roseae*
- *Gibbsiella gregii* (USA)



**Are *Brenneria goodwinii* and *Gibbsiella quercinecans* pathogens?**

**Contemporary Koch's postulates**



Modern adaptation of Koch's postulates fulfilled for *Brenneria goodwinii* and *Gibbsiella quercinecans* in 2018  
Combined traditional plant pathology methods with 'omics'

**Tissue necrosis within AOD**

- Necrosis caused by a poly-species complex or pathobiome
  - Two bacteria proven to cause necrosis
    - *Brenneria goodwinii*
    - *Gibbsiella quercinecans*
- Necrosis amplified when beetle larvae present!*

THE EMERALD ISLAND JOURNAL

**ORIGINAL ARTICLE**  
**Microbiome and infectivity studies reveal complex polyspecies tree disease in Acute Oak Decline**

Sandra Denman<sup>1</sup>, James Doonan<sup>1</sup>, Emma Ransom-Jones<sup>1</sup>, Martin Broberg<sup>1</sup>, Sarah Plummer<sup>1</sup>, Susan Kirk<sup>1</sup>, Kelly Scaife<sup>1</sup>, Andrew R Griffiths<sup>1,2</sup>, Maria Kaczmarek<sup>1,3</sup>, Jack Forster<sup>1</sup>, Andrew Pease<sup>1</sup>, Peter N Golshiri<sup>1</sup>, Francis Hassard<sup>1</sup>, Nathan Brown<sup>1</sup>, John G Kenny<sup>4</sup> and James E McDonald<sup>1</sup>

<sup>1</sup>Forest Research, Centre for Forests and Climate Change, Farnham, UK; <sup>2</sup>School of Biological Sciences, Bangor University, Bangor, UK; <sup>3</sup>School of Ocean Sciences, Bangor University, Bangor, UK; <sup>4</sup>Department of Computational and Systems Biology, Rothamsted Research, Harpenden, UK and <sup>5</sup>Centre for Genomic Research, Institute of Integrative Biology, University of Liverpool, Liverpool, UK

Decline-diseases are complex and becoming increasingly problematic to tree health globally. Acute Oak Decline (AOD) is characterized by necrotic stem lesions and galleries of the bark-boring beetle, *Agrilus lignatulus*, and represents a serious threat to oak. Although multiple novel bacterial species and *Agrilus* galleries are associated with AOD lesions, the causative agent(s) are unknown. The AOD pathosystem therefore provides an ideal model for a systems-based research approach to address our hypothesis that AOD lesions are caused by a polymicrobial complex. Here we show that three bacterial species, *Brenneria goodwinii*, *Gibbsiella quercinecans* and *Rahnella victoriana*, are consistently abundant in the lesion microbiome and possess virulence genes used by classical phytophagous that are expressed in AOD lesions. Individual and polyspecies inoculations on oak logs and trees demonstrated that *B. goodwinii* and *G. quercinecans* cause tissue necrosis and, in combination with *A. lignatulus*, produce the diagnostic symptoms of AOD. We have proved a



# Investigating a new serious stem canker disease in oak

Bridget Crampton, Forest Research



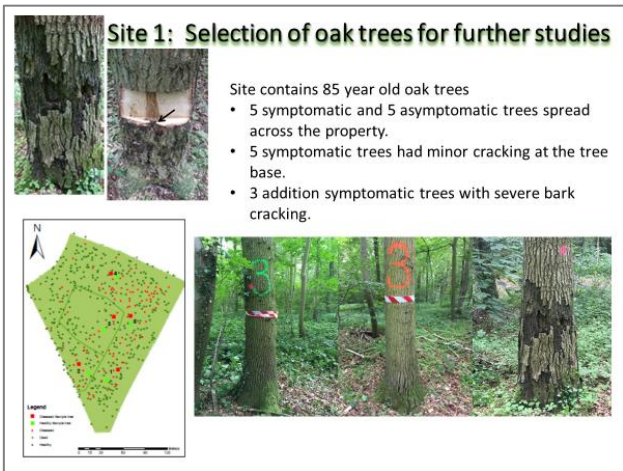
**Investigating a new serious stem canker disease in oak**

Bridget Crampton  
Sandra Denman

### Site 1: Selection of oak trees for further studies

Site contains 85 year old oak trees

- 5 symptomatic and 5 asymptomatic trees spread across the property.
- 5 symptomatic trees had minor cracking at the tree base.
- 3 additional symptomatic trees with severe bark cracking.



### Isolation of microbes from symptomatic and asymptomatic trees

**Buttress roots**

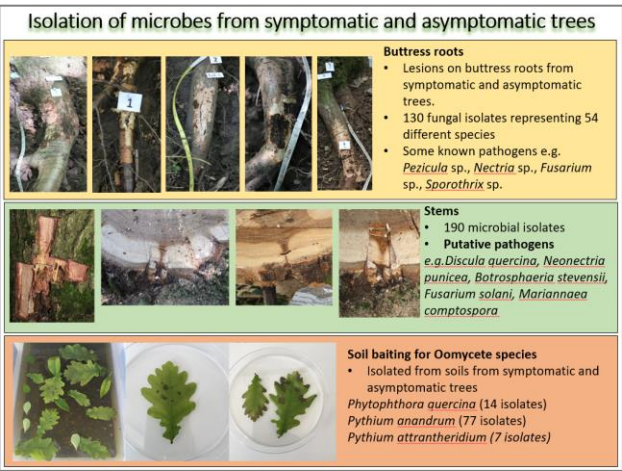
- Lesions on buttress roots from symptomatic and asymptomatic trees.
- 130 fungal isolates representing 54 different species
- Some known pathogens e.g. *Peticularia* sp., *Nectria* sp., *Fusarium* sp., *Sporothrix* sp.

**Stems**

- 190 microbial isolates
- **Putative pathogens** e.g. *Discula quercina*, *Neonectria punicea*, *Botryosphaeria stevensii*, *Fusarium solani*, *Mariannaea comptospora*

**Soil baiting for Oomycete species**

- Isolated from soils from symptomatic and asymptomatic trees
- Phytophthora quercina* (14 isolates)
- Pythium anandrum* (77 isolates)
- Pythium attrantheridium* (7 isolates)



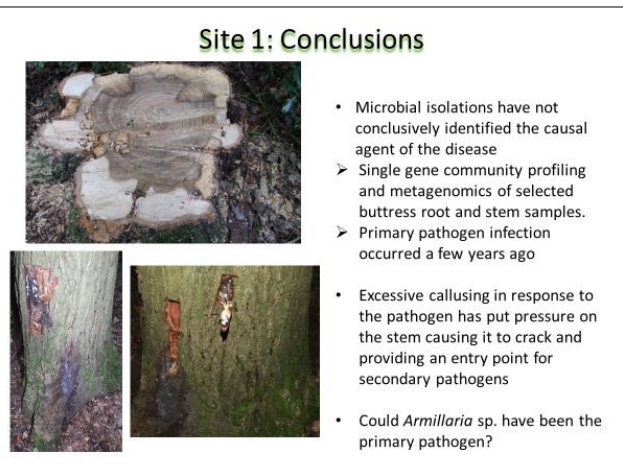
### Testing selected microbial isolates for necrotic lesion formation on *Quercus petraea* logs

*Mariannaea comptospora*    *Fusarium solani*    *Botryosphaeria stevensii*




### Site 1: Conclusions

- Microbial isolations have not conclusively identified the causal agent of the disease
- Single gene community profiling and metagenomics of selected buttress root and stem samples.
- Primary pathogen infection occurred a few years ago
- Excessive callusing in response to the pathogen has put pressure on the stem causing it to crack and providing an entry point for secondary pathogens
- Could *Armillaria* sp. have been the primary pathogen?



### Site 2: Disease symptoms on oak in Staffordshire



- Positive for *Phytophthora* sp. with a lateral flow device.
- Isolated and identified *Phytophthora cambivora* from lesions of diseased trees.
- Tree bleeds also positive for bacteria associated with AOD

## **Oak decline and ectomycorrhizas**

Laura M. Suz<sup>a</sup> and Martin I. Bidartondo<sup>b</sup>

a Kew

b Imperial

Like most temperate forest trees, oaks do not have fine roots (i.e. "feeder" or "absorbing" roots), they have ectomycorrhizas. These trees' fine roots do not interact directly with soil, instead tree interactions with soil nutrients, water and microbes are mediated by fungal tissues (i.e. mantle and Hartig net) that fully sheath the fine roots' surface and grow in between their epidermal cells. To understand how oaks interact with soils regarding nutrient and water uptake, and protection from attackers, we need to understand their mycorrhizas. We know very little about the link between functional diversity changes in ECM communities and oak health; this project represented a great opportunity to address this knowledge gap. Our aim was to test for links between environment, oak health and mycorrhizal communities. We sampled mycorrhizas from 189 trees in nine oak woodlands and parklands (Eastnor, Hatchlands, Richmond Park, Sotterley, Langdale, Attingham, Winding Woods, Great Monk Woods and Chestnut Woods) where some trees were showing symptoms of oak decline (acute – AOD or chronic – COD) while other trees remained healthy or in remission. We collected ca. 2,000 mycorrhizas and identified 206 different mycorrhizal species associating with oak in these sites. We found from 20 species in Winding Woods up to 81 in Hatchlands. On average, we detected 4-5 ectomycorrhizal fungi in the roots of each tree, independently of their health status. We did not find differences in richness, taxonomic or functional composition of mycorrhizal fungi across trees with different health status, but we found that trees showing AOD symptoms associated with more fungi with limited soil exploration abilities. However, when we compared the mycorrhizal fungi in these nine sites with 22 oak sites across Europe including Britain (Suz et al. 2014. *Mol. Ecol.* 23: 5628-5644) where there are no symptoms of tree decline, we found that their mycorrhizal communities differ. Further investigations are needed to 1) directly link fungal and tree health data with environmental data, 2) compare diseased and healthy sites, and 3) experimentally test cause-effect relationships between mycorrhizal changes and oak decline.

## **Phenotypic index measures for oak decline severity**

Jasen Finch, Aberystwyth University

Oak decline is becoming increasingly prevalent along with the need for accurate and quantitative oak health monitoring. The current methods for manually classifying oak decline status are often inaccurate due to the many visual characteristics that contribute to the definition of a declining oak.

We have developed the Phenotypic Decline Index (PDI) and the Decline Acuteness Index (DAI) measures to quantitatively describe and differentiate acute and chronic oak decline severity that represent the oak decline spectrum encountered in the field. These use a multitude of easily measurable visual phenotypic descriptors which include aspects of tree shape and size, crown condition and the presence of biotic agents such as bacterial stem bleeding.

The indexes were derived using machine learning approaches and provide simple but sensitive measures of tree decline severity. They enable the comparison of the visual health of oak trees both at different geographical locations and over time. These index measures have the potential to provide a useful tool for forest monitoring that could be used to inform forestry management strategies.

## **Chemical ecology approaches to manage oak declines**

József Vuts<sup>a</sup>, Philip J. L. Gould<sup>a</sup>, John C. Caulfield<sup>a</sup>, Sandra Denman<sup>b</sup>, Michael A. Birkett<sup>a</sup>

<sup>a</sup> Department of Biointeractions and Crop Protection, Rothamsted Research, Harpenden, UK

<sup>b</sup> Centre for Ecosystems, Society and Biosecurity, Forest Research, Farnham, UK

The biotic component of acute oak decline (AOD) is characterised by the co-occurrence of necrogenic microbial communities and larval galleries of *Agrilus biguttatus* (Coleoptera: Buprestidae) within the bark of native UK oak species. Our studies identified volatile small lipophilic molecules (SLMs) from live leaf and bark tissues of oak with behavioural and electrophysiological activity on *A. biguttatus*, which assigns to them the likely role of guiding host-seeking beetles to oak trees.

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 herbivores for feeding and ovipositing, but also by members of other trophic levels, i.e. parasitic wasps as natural enemies of *A. biguttatus* that seek host larvae for egg-laying. Similarly, the necrogenic bacteria that co-occur with *A. biguttatus* larvae under the bark, may have a significant effect on multitrophic interactions within this community. Although much is yet unknown of these relationships, the proposed complexity offers opportunities for further controlled laboratory and field trials to identify key semiochemicals (behavioural-modifying chemicals) that underline ecological connections.

We are using dynamic headspace sampling (air entrainment) to collect volatile SLMs, coupled gas chromatography (GC)-electroantennography (GC-EAG) to locate bioactive peaks in air entrainment extracts, GC-mass spectrometry (GC-MS) to identify bioactive compounds, multivariate statistical analyses to uncover chemical patterns linking multitrophic interactions, and laboratory bioassays and field trapping trials to determine the behaviour of *A. biguttatus* in response to odour blends and to identify volatile signal compounds diagnostic of AOD. Our research will underpin the development of semiochemical-based management tools, such as monitoring traps and chemical sensors, to mitigate the impact of AOD.

## **Monitoring oak tree decline using metabolomics**

Jasen Finch, Aberystwyth University

An exciting frontier is the study of the oak metabolome, the molecular building blocks of an oak tree. This has the potential to provide systemic chemical markers that could give indications of the overall health of an oak tree, long before visible symptoms become apparent.

To investigate this potential, phloem and sapwood tissues have been sampled from chronically declining oak trees at Chestnuts Wood in the Forest of Dean. The sample metabolomes were analysed using a multi-platform approach with high-resolution mass spectrometry technologies for broad metabolome coverage.

Oak decline severity was found to have a strong influence on the metabolome composition of the phloem and sapwood tissues that were validated across multiple years and in the wider woodland population.

Subsequent monitoring of the visual health of these trees can identify those that transition into decline. These trees could be used to identify predictive chemical markers related to the predisposing factors associated with oak decline. These could provide an exciting prospect for both monitoring and forecasting oak decline the individual tree and woodland scales.

Chemical markers such as these are likely to have great utility in informing forestry management practices.

## Population genomics of *Quercus robur* and its hybridization with the sympatric *Q. petraea* in British parklands

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The two predominant oak species in Europe, *Quercus robur* and *Q. petraea*, provide an interesting model to study interspecific differentiation as despite their clear morphological differences and different soil preferences they coexist along a gradient of ecological conditions and are interfertile. Hybrids are difficult to identify morphologically as individuals can show either an even mixture of traits from both species but often resemble just one species.

In this study we sequenced (23X) the whole genome of 386 oak trees, predominantly *Q. robur*, from four British parkland sites. We used this data to analyse the population structure of this species and its hybridization with, and differentiation from, the closely related *Q. petraea*.

Our genomic analyses show that there is no clear differentiation in the nuclear genomes of *Q. robur* among the four parkland sites. A stronger and more geographically correlated genetic structure was found in the plastid network. Nuclear genome data analyses were able to identify pure *Q. robur*, pure *Q. petraea* and a few admixed individuals. These analyses confirmed that the nuclear genomes of these two species are largely permeable.

Our whole chloroplast genome analyses confirmed previous work showing a lack of species-specific chloroplast markers between *Q. robur* and *Q. petraea*. Our chloroplast data revealed that the majority of the sampled oaks descend from a previously hypothesised lineage B, which originated in an Iberian Peninsula glacial refugium, though a minority were from lineage A. Altogether our plastid network reflects two distinct routes of oak colonization into Britain.

### Using metagenomics to identify leaf bacteria at sites with Acute Oak Decline

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Acute Oak Decline is known to have a polybacterial cause. Bacteria have been cultivated from stem bleeds and their genomes sequenced and submitted to NCBI. However, it is not known whether these bacteria are found elsewhere on the tree, or whether they are only found in AOD affected trees. We have a data-set of whole genome sequences of leaf and bud material from 453 trees in AOD sites. From these, we separated out genomic reads that did not belong to the host oak tree. These reads were then queried against a database of microbial genomes created from the NCBI refseq database using kraken software. The resulting kraken reports were queried for each of the AOD bacteria and each sample was scored for presence or absence of each bacterium. Evidence of the presence of *Gibbsiella quercinecans*, *Brenneria goodwinii* and *Lonsdalea britannica* was found across all 5 sites and on trees with and without AOD and in remission. This research is part of an ongoing PhD project and we hope to report in detail with a publication at a later date.

## **Monitoring the health of oak woodland**

Nathan Brown, Woodland Heritage

Trees are facing ever increasing threats from pest and diseases, with a changing climate is also enabling endemic species to have increased impact. In this moving context, monitoring is an important first step in detecting problems and mitigating their impacts. For Oak in Great Britain, modelling studies suggest that future climate scenarios will have varying affects on oak growth across the country, with areas of improved yield in the uplands, but worsening suitability in southern England. Empirical data are needed to assess the validity of these predictions and ensure that current trends are understood, with long term datasets vital to objectively assess change.

In 2019, a new initiative under the direction of “Action Oak” has set out to assess the underlying health of oak at a national scale. This work will use 85 ICP Forests level 1 sites (previously monitored from 1989 to 2007) as its foundations and aims to couple detailed phenotypic descriptions with modern analytical techniques. This dataset will provide a detailed description of the underlying health of Great Britain’s oak.

Across southern and central England an emerging complex disease, Acute Oak Decline (AOD), is of particular concern. Detailed monitoring of sites affected by AOD has taken place annually for the last 10 years, with 1200 trees assessed across 8 sites. This work has revealed important aspects of symptom development and indicated a predisposing role for environmental factors. 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. Similar patterns can be seen in the number of D-shaped emergence holes, although interestingly peak years are not always synchronised. We are currently investigating the mechanisms that drive AOD outbreaks focusing on how the number of symptomatic trees correlates with weather patterns.