Acute Oak Decline (AOD) Newsletter May 2017





Introduction

by Sandra Denman

As I begin this newsletter I muse on how seamlessly my annual AOD update has become a biennial event. This is not surprising considering the exponential increase in the number of Woodland Heritage funded PhD students and Post-Doctoral Researchers that have joined the AOD team over the past two years; the effort, time and energy it has taken to get them on the go has meant that other things receive less attention. However, the growth of the team is heartening and is largely due to the monumental passion, enthusiasm and drive Peter Goodwin put into raising awareness and funding to support AOD research, but is also due, in part, to the recently received Defra FPPH grant (Future Proofing Plant Health, £700K to carry research forward until 2018/19 FY).

Peter Goodwin, a great champion of Oak Decline research, passed away on the 18th of March 2017. Underpinned by the sad and poignant loss of such an extraordinary man, a great man, and a special friend to Oak trees, this report is a tribute to Peter and his quest for 'putting something back'. The four main frontiers of Woodland Heritage sponsored AOD research concern (a) the causes of the weeping lesions, where research on bacterial causes has been the main focus; (b) investigation of the role of the native buprestid Agrilus biguttatus in AOD, the drivers of beetle behaviour, and the potential for sensitive management; (c) the spatial epidemiology, spread and risk posed by the disease, and modelling management scenarios; and finally (d) through cross-linkage with Defra FPPH funded research at Forest Research, investigation into predisposition of Oak, which is all about what makes trees susceptible to disease in the first instance, and the early detection of trees becoming stressed leading to susceptibility.

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Sandra Denman with Peter Goodwin recording the voice over for the Woodland Heritage film on AOD research

Progress in Bacterial Research

We now have what we believe is robust scientific evidence showing that the key bacteria, Brenneria goodwinii (Bg) and Gibbsiella quercinecans (Gq), are able to decompose Oak tissue and cause lesions. Furthermore, we found that when bacteria are inoculated together with eggs of Agrilus biguttatus (which hatch and become larvae that chew sinuous galleries in the Oak innerbark), the lesions are bigger, and the bacteria can be isolated along the larval galleries. The tissues along the sides of the galleries and beneath them also become necrotic, forming part of the lesion. This suggests a role for the larvae in the spread of the bacteria inside trees. We have written up our results on the necrogenic action of key bacteria in the AOD complex (Bg and Gq), and submitted the manuscript for peer review. This is the most stringent test that scientific work undergoes before results are accepted as fact. We should get feedback within the next couple of weeks but in the interim we can share some of our findings with you.

We used a multifaceted approach to prove a necrogenic role for Bg and Gq, the key bacterial species closely associated with AOD. This means we did not view things separately but tried to understand and replicate how they operate together. We used both conventional log pathogenicity tests, as well as a suite of phenotypic (lab based assays) and genomic analyses. The rationale behind the work was that if a bacterium is able to decompose live Oak tissue and cause necrosis, the ability to produce the chemistry to perform this function will be evident in the genome of the organism. The genome is the total DNA of the organism, which holds the blueprint of what an organism should be capable of doing. Thus a key piece of research was to sequence whole genomes of key AOD bacteria Bg, Gq and Rahnella victoriana (Rv) to search for pathogenicity and virulence genes. This work was carried out by Woodland Heritage funded PhD student, James Doonan, who has recently been awarded his PhD (see feature article). James was guided and supervised by Dr James McDonald, at Bangor University. James Doonan discovered that the two key bacteria (Bg and Gq), as well as Rv, contained multiple pathogenicity genes that these bacteria could use to decompose Oak tissue.

In addition to whole genome sequencing, which identifies the *potential* an organism has to cause necrosis, it is vital to find out whether the genes are 'turned on/up regulated' and are thus functional. To address this we used two approaches. (1) Under controlled laboratory conditions various Oak tissues were inoculated with the bacteria. After certain incubation times the activity of the bacteria was measured. Results showed clearly that the destructive genes were activated in the presence of Oak tissue. James found abundant and active Plant Cell Wall Degrading Enzymes (PCWDEs), which macerate Oak cell wall polysaccharides by enzyme action. We were able to identify and home in on specific PCWDEs, for example pectinases, cellulases and tannases, and in laboratory assays showed that bacterial species possessing these genes were able to digest pectin, cellulose or tannin. For example, Gq showed intense tannase activity but Bg did not. Tannin is a host defence compound used by Oak and with Gq able to utilise this compound, it possibly clears the way for Bg to operate in other ways; so first insights into the synergistic interactions amongst these microbes are also being acquired.

We are aware that there could be other bacteria involved in lesion formation, as well as what might be considered as benign, ubiquitous fungi. To try and capture information about this, a Woodland Heritage funded Post-Doctoral 'Multi-omics' project was awarded to Dr Martin Broberg. Martin was based at Bangor University under the direct supervision of Dr James McDonald. (2) Thus, the second approach we took to solving causality of the weeping lesions characteristic of AOD was to interpret field data using advances in DNA sequencing technology. We



Martin Broberg sampling an AOD lesion for metagenomic and metatranscriptomic analyses

studied the genome (DNA), the transcriptome (the RNA (functional) copies of the expressed gene that are used to produce the gene products – i.e. proteins), and the proteome (the product encoded by the gene – e.g. enzymes) of the micro-organisms associated with the host.

Martin studied the genomes of the total microbial population (called the metagenome) associated with healthy, and AOD-affected trees at different stages of the syndrome. Metagenomics informs us about which microorganisms are present, and what genes they have. Results of the compositional microbial profile were consistent with the isolation studies and metabarcoding work contributed by Sandra Denman and her team, as well as by Melanie Sapp and John Elphinstone (in a previous Defra project). However, all these studies indicated that there are still a number of novel bacterial species not yet named but associated with the AOD microbiome that could influence lesion formation. Scientists at UWE are now making headway in sorting out the identities of some of these bacterial taxa.

In AOD lesions, when a gene becomes active (up regulated) a RNA copy of the gene (DNA) is made (thus RNA is an indicator of gene functionality). This RNA is then translated to form a protein (enzymes and/or toxins), which enable the bacterium to perform the function encoded by the gene. Thus 'metatransciptomics' tell us which of the genes are being used by the bacteria at different stages of the syndrome, and 'metaproteomics' identifies the specific destructive compound / gene (e.g. an enzyme) and tells us what it does. Lesions on field samples were analysed using 'metagenomics', to obtain evidence of genes present. Results showed that there were many genes present in AOD lesions. By careful mapping of the genes in the bacterial genome identified by James Doonan, with the information obtained by Martin from field lesions, we were able to cross-link some of the genes to show from which microorganism the destructive proteins originated, thus providing more evidence of the activity of these bacterial species. This work is ongoing but great progress has been made.

To supplement the above studies using different techniques, Carrie Brady from the University of the West of England (UWE), Bristol, has been investigating the interactions between frequently isolated bacterial species associated with AOD. Together with supervisor Dawn Arnold, she developed a quick and basic screening method to determine if AOD-associated bacteria can cause a plant response using green bean pods. A positive response indicates that the bacteria have pathogenic capability. Using this method the team at UWE has observed that a greater plant response is produced when certain bacterial species are combined with each other, rather than on their own. This research suggests that there is a reason why particular combinations of bacteria are routinely isolated from symptomatic Oak and it sheds light on possible synergistic interactions between bacterial species. Carrie is now on maternity leave for a year, but co-funded Woodland Heritage – UWE PhD student, Victoria Bueno-Gonzalez, will continue with some of this work, in addition to taxonomic clarification of novel bacteria associated with AOD.

During previous isolation studies, at least twenty-eight different strains, potentially belonging to the family Pseudomonadaceae were isolated from healthy and symptomatic Oak (FR). Commonly occurring strains were handed to UWE, where Victoria is carrying out phenotyping and genotyping analyses to reliably determine the phylogenetic position of the strains. Studies indicate that several additional possible novel bacterial species, some belonging to the family Pseudomonas, and some probably even belonging to new genera are associated with AOD in Britain. According to results obtained so far, at least three well-defined taxonomic groups, (A, B and C) could be identified. Clusters A and B are included in the P. aeruginosa group (which does contain plant and animal pathogens), while group C seems to be part of the P. stutzeri group. This work has been achieved in a record time of about six months. Once the identities are formally accepted, the metabarcoding, metagenomic and isolation data will have to be revisited to find out whether any of these novel species are uniquely associated with diseased tissue, and tests to determine whether they could have a role in lesion formation will have to be carried out.



Victoria Bueno-Gonzalez PhD student working on AOD bacterial taxonomy and pathogenicity at UWE

Progress in Agrilus biguttatus Research

Emma Bonham was the recipient of a Woodland Heritage funded PhD on 'Exploring the relationship between AOD bacteria, insects and the woodland environment'. Currently in its first year, this project is exploring relationships amongst three of the bacteria species (Bg, Gq and Rv) isolated from the stem bleeds of trees diagnosed with AOD, insects associated with declining Oak trees, and the wider woodland environment. The project has an overall objective of investigating potential pathways of transmission of the bacteria.

Two avenues of investigation are underway. In order to find out if insects could be acting as a vector for key AOD bacteria we want to establish whether any carry the bacterial species associated with the syndrome. The second aim is to investigate potential sources or reservoirs of these bacteria outside of the characteristic AOD lesions, for example elsewhere in the Oak tree itself or in the wider woodland. As the first year comes to an end this project has produced some interesting initial results. To date none of the bacterial genera have been detected within the various Oak-associated insect species investigated. Analysis of leaf, acorn and bark samples is still underway. Entering into its second year, a busy field season lies ahead with an expanded programme of sampling of both insects and the woodland environment for molecular analysis.



Emma Bonham inserting insect traps in Oak trees

Katy Reed is finishing her PhD study on the life cycle of Agrilus biguttatus. Katy has recently submitted an article for peer review on her research on the lifecycle of A. biguttatus, which includes data on the beetle's sex ratio, longevity, and fecundity, and the effects of temperature on its developmental biology. Her work suggests that the distribution of the insect is likely to be limited in the UK by its temperature requirements. An implication of this is that if the presence of the beetle is a necessary prerequisite of AOD, then the syndrome will be similarly limited by temperature. The data generated have already been used to produce a risk map of where A. biguttatus will occur (see fig. 1), and as there appears to be a very high co-occurrence of the beetle with AOD, this is important data to help predict where AOD will likely occur. The data will also be used to model how the beetle is likely to spread under a warming climate, and how this may influence the distribution of AOD and the risk it poses.

Predicted area suitable for A. biguttatus



Figure 1. Map of the predicted distribution of Agrilus biguttatus in England and Wales based on thermal requirements for survival of the insect. The red areas represent the thermal requirements of Agrilus biguttatus presence, other colours indicate areas that are cooler and less suitable for the beetle. The black dots represent the known occurrence of AOD in 2014

Katy also investigated Oak tree healing response as an indication of tree health status. This could be important in the biology of *Agrilus* because host defences may control



Figure 2. Callusing ability of Oak wounded by removing a 1 cm plug of tissue to the depth of the sapwood. A: Just wounded; B: Incomplete callus formation after 24 months; C: Wound closure and complete callus formation

the success of beetle development. This work explores the observations that although the larval feeding galleries of *A. biguttatus* are found beneath the bark of nearly all of the AOD-affected Oak trees examined, the D-shaped adult exit holes are only found on approximately 1/3 of affected trees. This may be because, although the trees have attracted egg-laying females, which are thought to be drawn to weakened trees, the host defences on 2/3 of trees with AOD symptoms remain too robust for the larvae to fully develop.

To measure trees' host defences, Katy monitored their ability to cover over wounds with callus-like tissue. Wound closure is an important defensive ability in AOD - trees in remission must cover over lesions to try and limit their spread – and it may also be an important defence against A. biguttatus larvae. If a trees' ability to callus over wounds restricts beetle development, AOD affected trees without exit holes should have a higher rate of wound closure than trees with exit holes. She wounded trees in different stages of Decline - asymptomatic of AOD, in remission, newly 'bleeding' from lesions, and those which had been bleeding for a number of years. She also categorised them according to whether they exhibited D-shaped beetle exit holes, and photographed the wounds regularly over two years, and compared the extent of wound closure between Decline stages.

Preliminary results show that, as expected, trees' ability to callus over wounds differed between the stages of Decline. Trees that had bleeding lesions for a number of years were least able to cover over wounds. The relationship between wound closure and exit holes was less clear. Sometimes, trees with exit holes had a higher percentage of wound closure than trees without exit holes. These results suggest that trees on which *A. biguttatus* has completed its lifecycle aren't necessarily that much weaker than trees in which it

hasn't completed its lifecycle, at least as measured by callusing ability. Other host defences, such as defensive chemicals, may be more important in resistance against beetle larvae. External factors, such as local temperature, may also influence the beetle's ability to complete its lifecycle on trees with AOD.



Daegan Inward and Ed Eaton taking narrow cores from a mature Oak for dendrochronology studies in relation to Agrilus biguttatus and AOD research

Chemical ecology of Agrilus biguttatus

Jozsef Vuts is also continuing with chemical ecology research on *A. biguttatus*. The rationale behind Jozsef's research is as with many other pest insects, a detailed understanding of how *Agrilus* detects the scent of the host Oak trees, which is crucial to develop environmentally benign management tools.

Initial chemical ecology research on the spread of AOD included a study on the attractant volatiles of the Oak jewel beetle. Using a combination of volatiles collected from leaves and bark, analytical separation techniques, electrical recordings from beetle antennae and highly sensitive identification tools, the chemical ecology team successfully identified volatile compounds from Oak leaves and bark that help guide the beetles to their host tree for mating, feeding and egg-laying. Since this initial discovery, their preliminary studies have gone on to provide further exciting results. Using laboratory behavioural experiments with adult beetles, evidence for the presence of a femaleproduced volatile sex pheromone that attracts males has been detected. This could potentially be utilised as a natural enemy (parasitoid) location cue against this Oak pest. Additionally, using the antennae of the beetles as a biosensor, Jozsef has shown that adults can detect specific compounds present in the volatile bouquet collected from the key pathogenic bacteria species found in the necrotic tissues of AOD-symptomatic trees. These results indicate the presence of a truly unique insect/pathogen relationship mediated by volatile compounds. Finally, the chemical ecologists have shown in the field, as well as in the lab using Oak logs infected with a combination of AOD-associated bacteria and Oak jewel beetle eggs, that AOD infection appears to boost the production of certain Oak volatiles. This suggests a complex signalling interaction between the host tree, the pest beetle and pathogenic bacteria via upregulation of genes involved in Oak tree metabolism. By taking forward these tantalising discoveries, there exists a unique opportunity to elucidate a host-pest-pathogen complex that severely affects UK Oak populations, and provide the underpinning science for management of AOD using novel chemical ecological tools.



Figure 3. AOD infection induces the emission of certain leaf stress volatiles. Asterisks mark the two terpene compounds, the emission of which was consistently elevated both in field and laboratory experiments

Progress in Spatial Epidemiology Research

2016 was a busy year. It marked the completion of the Defra funded TH0108 project of which the AOD national survey was a major component, and there was much to write up in the wake of the research results. The main aims were to estimate the boundaries and extent of AOD, severity/incidence for certain sites, and to predict the risk and likely drivers of the spatial spread of AOD in Britain. The key findings are that AOD positive sites are located in similar areas to the reports from the public.

This gave confidence that the survey area did not need to be extended to Scotland. The survey did however expand the known range, with a new northerly positive near Sherwood Forest (close to Worksop), and more surprisingly, showed that AOD is present in the south west across Wiltshire and Devon. This suggests either recent spread, or more likely, under-reporting in the region. Nonetheless, public reports were shown to cluster around



Nathan Brown training Andy Griffiths how to use the Trimble, an accurate GPS that enables the mapping of tree position to an accuracy of 10 cm. This apparatus is used to generate maps of the trees in our study sites

affected sites in the survey selected squares, reinforcing the idea that citizen reported data is representative and can be useful in predicting the area affected by AOD. However, the citizen reported data showed regional biases and did not occur at the same intensity as survey detections. Overall, we welcome and encourage the important input citizens make towards tracking the incidence and occurrence of AOD. Please continue to report cases to FR. Prediction maps were generated, one example of those is included here *(Figure 1, page 5)*. The thermal requirements of the *Agrilus* beetle were used to inform the model, but as more environmental information is gathered the AOD risk map may change.

In the autumn of 2016 a new Defra FPPH project was set up, and concurrently Woodland Heritage funded a Post-Doctoral study that aims to develop models to assess within site management of AOD, and to set up at least one large scale management trial. Nathan Brown leads this research and the intention is to work in an integrated, holistic way with other disciplines to formulate and assess management solutions to AOD and COD. Common to all research areas and underpinning all the projects, is the topic of predisposition to Decline. We are currently using the data from the national AOD survey to investigate links to environmental factors such as rainfall, temperature and chemical (pollutant) deposition. Early results have revealed some important correlations in national datasets, which will now be investigated directly within AOD affected Oak woodland.

Management of native woodland is often in the hands of private individuals whose aims and goals can vary widely, from non-intervention conservation to high forest timber production, and often include a mixture of interests. This makes understanding the management decisions vitally important when considering advice for Oak woodlands in Decline. In the coming year we plan to conduct a survey amongst woodland owners and managers to record perceptions to AOD and environmental change. For this to be useful it is very important that we can gather the opinions of a wide ranging group to be representative of all the different woodland users. Obviously the views of the Woodland Heritage members will be important to include in this process. It is our hope that you will support Nathan in this endeavour and will be willing to take part in the survey, which will likely be ready in the autumn/winter 2017.

Progress in Predisposition Research

Woodland Heritage has invested in investigating the metabolic status (metabolome) of AOD affected trees through awarding a three year Post-Doctoral study to Jasen Finch at Aberystwyth University, under the supervision of John Draper.

It is well known that Oak Decline syndromes are the result of interactions between predisposing, or stress inducing environmental factors such as drought and pollution, that lead to susceptibility to pathogen and insect pest attack. Metabolism forms the interface between the expression of an organism's genes and the environment in which it lives. In order for an organism to adapt to its environment and react to stressful conditions, it has to alter its physiology. This is achieved through changes to gene expression, which are then evident in the host metabolism. Comparative study of metabolic changes of healthy Oak with those under stress provides a valuable snapshot of the adaptations trees make when stress conditions prevail, and may be useful as early detection indicators of trees undergoing stress and becoming predisposed to biotic attack.

So far this study has produced a minimally destructive sampling protocol in which small arch punches can be used to sample innerbark (phloem) and sapwood tissue types. Only a small core (1cm diameter holes to a depth of approximately 3cm) of live Oak tissue provides enough material to allow multiple chemical analyses. Sampled trees are usually readily able to heal the wounds (see callus experiment by Katy Reed, page 6), minimising the damaging impact of wounding.

It is difficult to assess true tree health status on visual inspection alone. Our results show that rigorous, systematic examination of visible Decline symptoms and overall tree architecture is essential to select representative healthy and AOD symptomatic trees. A combination of more than 5% crown dieback, bleeding lesions and beetle exit holes was identified as a key indicator of AOD status. Information on historic annual tree growth (dendrochronology) is helpful in obtaining true health status of trees, but the process is invasive and therefore not practical on a large scale or when working on high quality timber trees.

Initial results on three sites show large variability in tree metabolomes, but a consistent range of metabolites in both innerbark and sapwood are clearly associated with AOD symptoms. The chemical classes of these metabolic compounds involve sugars, amino acids, polyphenols and alkaloids.

Sugars are important metabolites for transport, storage and use of carbon fixed by photosynthesis. A detailed analysis of starch reserves and the profile of transported sugars will be undertaken in the 2017 growing season to determine whether environmental stress leads to susceptibility to AOD through disrupted photosynthetic efficiency.

Furthermore, a number of chemicals (mainly phenolic and alkaloid) were found not only to have an association with the presence of AOD symptoms, but also with the degree of crown dieback present in symptomatic trees, and therefore potentially with the degree of AOD severity. Some of these chemicals have also been implicated in plants as both antifeedants against insect herbivores, and antimicrobials against bacteria and fungi. These data suggest that trees with a reduced content of such protective metabolites could be at higher risk from feeding damage by *A. biguttatus* or necrosis by pathogenic bacteria, for example Bg and Gq.

With standardised methods in place for the rapid sampling and analysis of the tree metabolome we are now well placed to determine whether any of the metabolic pathways predicted to be disrupted in AOD symptomatic trees, are perturbed in visibly healthy trees that are at risk of becoming predisposed to developing Decline syndromes based on epidemiological modelling.



John Draper and Jasen Finch using mass spectrometry to obtain metabolome fingerprints of healthy and diseased Oak

Partnerships with the Defra FPPH project

As pointed out above, host predisposition is fundamental to the onset of Decline, but evidence is limited and appears isolated, so that the linkages between Decline factors and host effects are poorly understood. Soils, pollutants and/or nutrients (particularly carbon and nitrogen), root health and the rhizosphere are important predisposing elements, and a key question that the FPPH project aims to address is whether there are 'specific predisposition factors leading to the development of Oak Declines in the UK'.



Elena Vanguelova collecting soil samples for analysis as part of the FPPH collaborative research effort

Building on the groundswell of current research and meshing with ongoing initiatives, the FPPH project will investigate relationships among biogeochemical factors (soils and leaves of Oak), rhizosphere microbiota, roothealth, historic tree growth and nutrient utilisation, and tree metabolic function. The research entails soil, root and foliage sampling to identify biogeochemical factors associated with Oak health. The soil and root microflora will be profiled to determine both beneficial (e.g. mycorrhizal) and detrimental components. The functional composition (active genes) of the root microflora will be assessed to quantify key genes (and putative sources of these genes) involved in nitrogen and carbon cycling. This will give insights into nitrification potential and root health and function.

Information acquired from analyses will be related to the incidence of disease on the ground, and this will lead to a greater understanding of the effect of environmental factors on host susceptibility to disease. The research aims to lead to development of enhanced preventative and restorative management advice that in turn leads to better soil and forest management practices that will produce resilient Oak trees. The complex nature of the problem requires a sustained and multidisciplinary effort to attain practical recommendations. Without the generous support of Woodland Heritage, other charities, Defra and the Forestry Commission, as well as the will to share, interact and co-operate among the researchers, landowners, practitioners and stakeholders, an undertaking of this nature would not be possible. We are truly grateful for this wonderful opportunity.



Tree climbers shinning up a mature Oak tree. They will be collecting foliage from the tree tops, which will be analysed for nutrient content. Results will be compared with the soil and root analyses. Overall the investigations will give insights into the health and functioning of the tree, and the status of the metabolism of the tree, which if out of balance, might be ameliorated by addition of essential elements

In conclusion

Peter Goodwin had the timely realisation that Britain's native Oak trees were under increasing pressures that were undermining their resilience, leading to deteriorated health and premature death. He was alarmed and very unhappy about this, but determined to do something about it. He felt that 'Doing nothing was the worst thing you could do'.

Peter immersed himself in raising funds so that research could guide preventative and restorative management, and safeguard Oak in the future. He travelled to woodland after woodland, seeing for himself, assessing the scope and extent of the problems facing Oak. He was a frequent visitor to the laboratories at Alice Holt where he kept up with the latest



This picture depicts a message of 'hope' and 'opportunity'. It is an Oak tree in a woodland once owned by Peter Goodwin. This tree had a massive AOD attack, but is now in remission, callusing over the once severely weeping, decomposing Oak tissue. The 'hope' part is that, given a chance, an Oak tree can overcome an attack of AOD and survive; the 'opportunity' aspect is that through research we will learn how to prevent AOD attacks in the first instance, but also how to manage the situation to give those trees that have been affected, the best chance possible of recovering

research developments, and was an ardent supporter of the work that was carried out there. His enthusiasm abounded; he was a great and genuine source of support and encouragement to the researchers. Peter understood the complexities of the problem, and knew just what it would take to make significant research progress. 'Action not words' is what Peter set out to implement. He was visionary and proactive in mobilising industry, stakeholders and government to meet and explore initiatives that would sustain research, to ensure continuity of knowledge and produce highly trained, skilled scientists. Peter was very keen on supporting young people, welcoming them to the industry and giving them every opportunity to get off to a flying start. Peter very definitely 'put something back'. He leaves an astounding legacy of commitment, and focus on the health of Oak, more than fifteen trained scientists, and a great general awareness of the challenges Oak face. But most of all, he leaves with us the inspiration and determination to bring the job to full fruition, and to ensure that Britain's native Oak trees will triumph, remaining our true heritage for future generations.



Nathan Brown and Mark Oram monitoring an AOD tree to understand the spatial epidemiology of AOD, which will lead to the development of management models on how to halt spread of the disease

Peter Goodwin's first encouragement to his fellow trustees of Woodland Heritage to act and not talk about addressing the threat posed by Acute Oak Decline (AOD) probably took place about a decade ago.

By 2009, Peter had raised the first funds that were spent on studying bacteria in Oak in a partnership with Forest Research and Imperial College, which also started the long-term involvement in this field of Nathan Brown, who continues to be a major force in the battle against AOD, now based at Rothamsted Research.

By 2010/11 Woodland Heritage-funded research was well underway at University of West of England, Bristol with Bangor University becoming a research partner just one year later; working relationships that under Dr Sandra Denman's overall guidance and supervision continue to flourish to this day.

The last newsletter was produced two years ago, by which time around £0.25m had been spent on research from funds raised by Peter Goodwin. This was a considerable achievement, yet in 2015/16 almost £0.25m was spent on research by Woodland Heritage and even more amazingly around £0.5m in 2016/17; for a couple of years it could be said that what had been spent that year matched what had been spent in all proceeding years.

The spending was reflected in the number of scientists paid for by Woodland Heritage able to supplement Forest Research's ongoing and separately funded work. For Woodland Heritage, 'peak research' took place between October 2016 and March 2017 when a dozen separate studies were underway, all supported by Woodland Heritage, with new universities now involved such as Aberystwyth, Harper Adams and Swansea, as well as Rothamsted Research. All of this amazing work on the ground that is reported in this newsletter was made possible by Peter Goodwin single-handedly raising £2m on behalf of Woodland Heritage to be spent on AOD research, a sum that continues to grow with many gifts being received after Peter's death and with profound respect for all that he had achieved.

As has always been the case, gifts made to Woodland Heritage to enable it to help boost research into AOD have come from many quarters, although since the last newsletter in 2015, particular thanks are extended to The Monument Trust, The J. Paul Getty Jr Trust and The Gatsby Charitable Foundation for their vital and timely interventions that were significant contributions towards the period of 'peak research'.

Other notable supporters have included Lord Sainsbury of Turville, The Rothschild Foundation and The Rufford Foundation, but it goes without saying that all gifts of whatever size are greatly appreciated by Woodland Heritage and all play their role in making a difference towards the better understanding of the significant threat posed by AOD to this nation's most iconic tree.

To all who have given and will give in the future, Woodland Heritage sends its most grateful thanks; we know we are in a marathon and not a sprint and we will need real stamina and resilience both from our team and supporters to succeed. However, we are not sure if future generations would forgive us if we didn't do all in our power to protect our trees, in particular our national tree, the Oak.

Lewis Scott and Guy Corbett-Marshall, Woodland Heritage

Woodland Heritage is a registered charity number 1041611



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