Summary Notes of the Oak Resilience Knowledge Exchange Event held at Alton House Hotel on the 27th February 2020

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Forest Research (FR) held a knowledge exchange event at the Alton House Hotel to report their most up-to-date research on ***Oak Resilience*** to stakeholders, funders and policy advisors and to raise awareness of their research in celebration of, and as a contribution to the international year of plant health (IYPH).

The meeting was attended by 73 people representing woodland and forestry charities / groups, national property charities, Forestry England, Natural Resources Wales, Scottish Forestry, Defra, landowners, land managers, foresters, consultants and scientists.

The meeting was opened by Dr Sandra Denman outlining the importance of the iconic British oak to the UK landscape, society and economy, the environment and biodiversity and highlighting the need to take a holistic, interdisciplinary approach to research and management. Britain's native oaks are facing increasing pest and disease threats and stress pressures. Direct links to increased global trade, high pollutant levels, disturbances, climate change, under- or unmanaged sites can be made. More needs to be done to ensure resilience of trees. 2020 Is a year specially designated by the United Nations as the IYPH, it is our duty and an opportunity to raise awareness and demonstrate our commitment to ensuring a secure future for oak and indeed all trees.

Oak Declines and the underpinning importance of environmental nutrient balance in tree health

Dr Elena Vanguelova explained the process and cycling of nutrients in forest and tree ecosystems. Both macro and micronutrients are essential for plant health and growth. Balance amongst the available chemical elements (nutrients) is essential for optimal tree health. Nutrient imbalances lead to compromised function, reduced growth and defence ability. Woodlands and treescapes in Britain face many challenges – there is much fragmentation in woodlands leading to smaller more isolated patches, altered forest microclimate, habitat change and species loss. Below ground there is also large spatial variability in soil types and drainage; and above ground the atmospheric chemical composition is undergoing changes. These changes can place trees under stress and predispose them to greater pest and disease risks. A desktop study was done to identify potential predisposition factors at the national scale, followed by field site and tree scale investigations to determine links between predisposition factors and root and foliage attributes. At the national scale there were correlations between warmer temperatures and low rainfall as well as low levels of dry (i.e. atmospheric gaseous forms of) sulphur and high levels of dry nitrogen. A risk map was made based on these findings. At the site level preliminary results indicate that two groups of potential predisposition drivers are correlated with (1) the presence of AOD and (2) the absence of AOD and presence of COD and 'apparently healthy' trees only. In the first case (1), which occurred in AOD trees, it appears that acidification of the bulk forest soils leads to a deceased uptake of essential nutrients such as nitrogen (N), phosphorous (P), calcium (Ca), potassium (K) and magnesium (Mg). This is exacerbated by changes to feeder root morphology such as fewer feeder roots, lower feeder root density and biomass, fewer root tips and low levels of root branching. Thus, there is an ***acidity driven scenario*** that leads to nutrient deficiencies and imbalances in the AOD symptomatic trees.

The second case (2) appeared to be on non-AOD sites where COD trees were present. The model showed low levels of soil N, P and K (in some cases), higher feeder root biomass and density, large numbers of feeder root tips and root branching occurred but there was lower uptake of N, P, Ca, Mg and K. COD symptomatic trees had smaller leaves, with both leaves and roots being deficient in N and P. Thus, it appears that there are key deficiencies in the soils and the trees are sending out 'exploration roots' in the search of the nutrients the trees require. The second case model thus appears to be a ***nitrogen and phosphorous deficiency driven scenario*.**

Oak feeder root health and functioning in relation to soil chemistry and microbiomes.

We know that microorganisms are crucial in nutrient availability and uptake for example through ectomycorrhizal fungi and in regulating soil nitrogen (N) by changing its chemical structure into forms that plants are able to use (nitrification), but microorganisms may also play key roles (positive and negative) in the abundance, type and functionality of feeder roots; for example a negative role would be rotting root tips off. Four studies were carried out to investigate this. In the first study, soil bacteria that convert N into plant available forms in the soil were investigated and it was clear that healthy trees were associated with a higher abundance of ammonia oxidising bacteria (AOB) than declined trees and that this relationship was strengthened in less acidic soil environments. This also thus confirms that the lower pH (more acidic) conditions in the AOD sites could be linked to the lower N uptake by the symptomatic trees. AOD sites had about 10 times higher ammonium NH_4 in soil (~15-30 g/kg) and nitrification potential compared to COD sites (~0.9-2.5g/kg). Soil available NH_4 releases H^+ ions when it is converted to NO_{3-} (plant available form of N), which contributes to making soil more acidic and thus resulting in lower levels of AOB at AOD sites. The lower soil NH_4 and nitrification potential at COD sites provides trees with less available NO_3 for uptake at COD sites. Thus, the two separate studies corroborated the two preliminary models. Much more work is required, and cause and effect mechanisms need to be understood but this information provides the first steps towards the possibility of chemical amelioration to obtain nutritional balances in tree soils.

The second study investigated the *root fungi* isolated from symptomatic and healthy *feeder roots* and the presence of Oomycetes (*Phytophthora* and *Pythium* species) in soils to find out whether there were fundamental differences in the feeder root mycoflora and to find out if any of these micro-organisms contribute to rotting root tips. Both traditional culture and molecular methods using single gene community profiling were used. Results are still being processed but preliminary culture-based studies indicate more than 50 genera of fungi are associated with oak feeder roots in the UK. At this stage there do not seem to be many differences between the most commonly occurring fungal communities across all the sites. There was a very high abundance of *Illyonectria*, which is a known feeder root pathogen, irrespective of the health status of the trees. A number of *Phytophthora* species were isolated from soils on the AOD sites but were not specifically linked to tree health condition on those sites. Only about 25% of the fungi isolated have been identified so far so these preliminary results may change and it is not possible to draw conclusions yet.

Dr Greg Deakin and Prof Xangming Xu carried out a study on the *microbial component of the rhizosphere soil* with the aims of finding out if there were microbial linkages between the microbial communities inside feeder roots and those in the rhizosphere soil and how these related to tree health condition. They studied the composition and function of the rhizosphere microbiome (i.e. the collection of all microbes in a particular environment). They used molecular methods to address the questions: (1) is there a difference in microbial diversity between healthy and diseased rhizosphere and (2) do the microbiomes differ in their gene and protein content. Similar to the isolation study, they found that there were no overall differences in rhizosphere microbial communities between

healthy and symptomatic trees, but there were significant differences in the relative abundance of specific taxa (species) between samples from healthy and symptomatic trees. There were very few common features in the different health condition of trees but there was a possible reduction of beneficial microorganisms at AOD/COD sites. In terms of the functional gene and protein they found numerous differences between diseased and healthy samples, but without clear interpretable patterns, and concluded that AOD or COD is not attributable to specific rhizosphere pathogens.

On the positive side, Dr Laura Martinez-Suz explained that oak trees do not interact with soil directly, but through their *mycorrhizas*, which are fungal filaments that sheath feeder roots and obtain carbon from trees in exchange for soil water and nutrients, which are taken up by the ectomycorrhizal fungal filaments that extend into the soil. Ectomycorrhizal fungi are therefore directly involved in oak survival and growth. To understand how oaks interact with soils regarding nutrient and water uptake, and protection from attackers, we need to understand their mycorrhizas. Across nine oak woodlands and parklands in UK, 206 different mycorrhizal species associating with oak were identified. There were no differences in richness, taxonomic or functional composition of mycorrhizal fungi across trees with different health status, but trees showing AOD symptoms were associated with more fungi with limited soil exploration abilities. However, comparing 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, it was clear that their mycorrhizal communities differ from those at our study sites and UK oaks might even be considered mycorrhizal impoverished. Further investigations are needed to 1) directly link fungal and tree health data with environmental data, 2) compare diseased and healthy oak sites, and 3) experimentally test cause-effect relationships between mycorrhizal changes and oak decline.

Dr Carrie Brady pointed out the importance of knowing the precise identity of the microorganisms involved when diagnosing diseases or assigning microbial functions for example in the nitrogen cycle. Without a formal name of a species accurate identification and diagnosis could not take place and authorities would not be able to create quarantine legislation or regulate movement of biological material/bacterial cultures. This would lead to the risk of tree pathogens being moved globally. Identifying bacteria is a multifaceted task but over the past 10 years Carrie has identified 14 novel bacterial species from AOD lesions and 5 sub-species and has created 2 new bacterial genera.

It is well known that the degradation of oak stem tissues characterising AOD are caused by a suite of bacterial species and that *Brenneria goodwinii* is mainly responsible. Dr James Doonan showed results of controlled experiments that demonstrated the genes that *B. goodwinii* and *Gibbsiella quercinecans* use to break Oak tissues down. The bacteria appear not to compete with each other but to complement each other. James analysed controlled log tests where bacteria and larvae of the *Agrilus biguttatus* beetle were inoculated into the logs either or not. Only about 400 genes were active when the bacteria were inoculated alone but this number increased 10-fold to over 4000 genes becoming active in the presence of the larvae. This suggests there is some trigger mechanism associated with the larvae activating the majority of destructive genes in *B. goodwinii*. Although this work is very expensive to do, more repetitions are required to prove the case categorically and either prove or disprove an essential role for the beetle in AOD.

Dr Nathan Brown described the importance of long-term monitoring Oak health. Previously in a European-wide initiative (the Forest Condition Survey) 90 plots across the UK were monitored. In 2006 this abated in the UK but continued in Europe. Nearly 15 years on Nathan has found the 90 plots and all the trees that were monitored and has reinstated the monitoring process. He has also

monitored the development of AOD very closely for the last 10 years. Clear patterns of epidemic surges are emerging in association with very hot dry weather years followed by wetter years.

During the monitoring process consistency of metrics used to judge the health condition of Oak became necessary. Dr Jasen Finch talked about a Phenotypic Decline Index (PDI) and the Decline Acuteness Index (DAI) he developed to quantitatively describe and differentiate AOD and COD severity using a number of easily measurable descriptors. Although some way off, this tool will be available as an App and so can be used by woodland managers, foresters or anybody with a mobile phone. As Oak declines are becoming increasingly prevalent it is increasingly important to have standardised, harmonised, objective measurements that can be compared over time, which will allow a reliable analysis of the true health of trees over time.

Jasen then went on to talk about his work on utilising metabolomic technologies for investigating potential systemic chemical markers in the oak phloem and sapwood tissues as indicators of oak health. He has found that oak decline has a strong influence on the chemical composition of these tissues in trees at a woodland in the Forest of Dean. These signatures have been validated across years and in the wider woodland population. Jasen also discussed the potential for identifying chemical signatures associated with predisposing oak decline factors that could have great utility in monitoring and forecasting oak decline in the future.

Along the theme of Oak characteristics Queen Mary College, London, PhD student, Gabriele Nocchi explained that he was applying whole genome sequencing (WGS) of the oak cell nucleus and chloroplast for two reasons. (1) To characterize the oak species and populations at the different study sites using oak nuclear DNA so that the populations could be compared and (2) characterize the chloroplast DNA to obtain insights to the evolutionary origin of the trees at the different sites. More than 380 trees were sampled from four study sites (two in the west and two in the east of England). Gabriele found oak species-specific markers that enable him to distinguish *Q. robur* from *Q. petraea* and also to separate out hybrids of the two species. This is an exciting development, which could have useful field application if a field test kit can be developed. The second aspect of the study revealed that 6 different oak groups (called haplotypes) could be distinguished at the four study sites. Most of the Oaks appear to have originated from the Spanish (Iberian) refugium but at one site in the west, Oaks from the Balkan refugium were also present. Oaks in the east appeared to have little genetic diversity, most appeared to be in haplotype 1. On the other hand, Oak from the sites in the west were genetically more diverse with representation of all six haplotypes.

Another QMCL PhD student Louise Gathercole presented her work on microbiome studies of the leaves collected for Gabriele's study. She set out to establish whether she could use WGS of leaf material to identify AOD causal species and to find out if the AOD bacteria could be found on both healthy and affected trees and whether there were differences in occurrence of the bacteria at the different study sites. Louise found that WGS could be used for this purpose and that the AOD bacteria were omnipresent at all sites irrespective of the health condition of the trees. It will be important to find out whether these bacteria exist in sites where AOD is not present.

Chemical ecologist Dr Jozsef Vuts spoke about proactive research for potential beetle management. Jozsef has found chemical signals that are attractive to *Agrilus biguttatus* beetles and he tested these in the filed using them as lures in different colour traps. He found the highest catches in green funnel traps with the lures inside. He was also able to catch a lot of parasitoid wasps that parasitise the beetles, using these traps, which is an exciting development for the potential of biological control. Jozsef also tested the beetle's response to volatiles given off by the AOD bacteria and found that the beetles were attracted to these as well. This means that he may be able to heighten the efficacy and specificity of the lures for the beetles.

The final two talks were given by senior FR entomologist Dr Daegan Inward and pathologist Dr Bridget Crampton. Daegan spoke about the Oak Processionary Moth (OPM) outbreak in the UK, explaining how and where it occurred. Defra (Lisa Smith) and Forestry Commission Head of Plant Health Dr Anna Brown had done a magnificent job of tracing all the trees and having them destroyed, but people were asked to be on the lookout for occurrences of OPM this year. He also mentioned the approaching threat of the Oak lace bug, *Corythucha arcuate*, a native American sap-sucking insect that has been introduced into central and eastern Europe causing leaf mottling and defoliation with impairment of photosynthesis, reduced growth and vigour occur. Also, of concern, are damaging wood and bark boring beetles. The PSHB, Polyphagous shot hole borer (*Euwallacea* sp), which is one of 3 cryptic species in the *Euwallacea fornicatus* complex, is native to Asia (e.g. Vietnam) and carries with it a fungal symbiont *Fusarium euwallaceae*, which colonises the larval galleries and acts as a food supply for developing larvae. However, the fungus is highly pathogenic to tree hosts causing vessel blockage, wilting and dieback. This disease is emerging as an important invasive pest killing avocado and other trees in Israel, California USA, and South Africa, and has an extremely high host range including Oak and plane trees.

Dr Bridget Crampton described her research on two stem canker diseases of Oak. In one case canker, basal bark cracking, outer bark flaking off the stem and callusing lesions on both buttress roots and the tree trunk occurs on mature and maturing oak (c >80 years). This work is still in progress and although a suite of known pathogenic fungi have been isolated including *Fusarium*, *Pezicula*, *Nectria* and *Sporothrix*, the microbial isolations have not conclusively identified the causal agent of the disease and single gene community profiling studies will be carried out. One hypothesis about the disease is that initial severe damage was caused quite some time ago and the agent is no longer present / active, and the bark cracking is associated with tree callusing trying to overcome the damage previously inflicted. In a second and different case extensive bark necrosis was found on young oak (c 45 years old). Symptoms of severe basal cracking and stem bleeding were characteristic and *Phytophthora cambivora* was considered the primary disease-causing agent, but interesting the AOD bacteria *Brenneria goodwinii* and *Gibbsiella quercinecans* were also isolated and may have a contributory role.

Overall the day was packed with knowledge transfer and networking. The scientists gleaned so much from exchanges with stakeholders, many new research questions have arisen, and stakeholders were impressed by the amount and scope of the knowledge amassed and the high-tech science underpinning it. Over the course of the day it was apparent that several themes arose. One theme is the need to continue with the research as many studies are breaking new ground in the health and resilience of UK oak and practical solutions are starting to come through. This was apparent in the soil, ectomycorrhizal and root pathogen work where risk mapping is improving with each step of the way, but we were concerned to hear that the ectomycorrhizal situation on our oak trees in the UK is so impoverished by comparison with the position in Europe. Another theme that emerged was the development of new and innovative tools that will have practical application to industry and diagnosticians. Exciting examples were Bridget Crampton's LAMP method to identify oak roots (or other tissue) from other species. Building on some of the work carried out by Gabriele Nocchi this could be further developed to a field ready Lfd test that could be used by practitioners. Jasen Finch has developed a model to standardise assessment of oak health, which will become available as an App on smart phones. The workshop demonstrated the advantages of interactive discussions, and finally long-term monitoring is essential to baseline the health status of our trees and to understand and proactively manage them so that they are resilient to the changes they face. We thank everyone for attending the meeting and for contributing to its success and invite everyone to enjoy the film documenting the day.