No decline in Oak research in 2021

by Sandra Denman (Forest Research)

It feels terrible to begin an article on a sad note, but the losses experienced in 2021 lie heavily on my heart.

To us all as Woodland Heritage supporters, the sudden loss of Lewis Scott in November 2021 has left us bereft of his elegant leadership and gentle humour. Opposite in nature to Woodland Heritage's Co-founder, Peter Goodwin, Lewis operated in a quiet manner, whereas Peter radiated dynamism, action, and charisma. They led a wonderful, inspired organisation in a balanced way. How we now miss both giants of our industry.

We are so grateful to them both for their vision and their drive to fund tree health research and draw together public-private funding partnerships especially through the Action Oak initiative. Without this endeavour and the generous support of Woodland Heritage members and its related charities, we would not be where we are in our understanding, leadership, and management of Acute Oak Decline (AOD). We sincerely thank them for all they did to help secure the good health and resilience of our Oak trees for future generations.

2021 was not all bad; challenging without doubt, but exciting in many ways as well.

The Bac-Stop project

Last year, I wrote about the UKRI (UK Research and Innovation) funded Bac-Stop project and described all the things we were '*going to do*'.

This year, I can tell you about some of what we have done and how we did it. I share with you information on cutting-edge tree technology that we are using to monitor how Oak trees physical and metabolic (chemical) functions are affected by environmental stresses and disease. I am finding this part of my research journey exceptionally exciting. I am learning so much from working side-by-side with my colleague, Dr Elena Vanguelova (Figure 1), who heads soil and biogeochemical research at Forest Research (FR), Alice Holt, and is the lead on the soil, environment, and tree physiological aspects of our field trial.

Figure 1: *Dr Elena Vanguelova, Senior Soil Biogeochemist at Forest Research, taking soil samples at the Bac-Stop field trial*

We set out a field trial based on the premise that *predisposition* has a fundamental role in Oak Decline. *Predisposition* is a condition brought about by both biological and environmental stresses that weaken trees. It leads to ripple effects that cause deterioration of tree health, which often ends in tree death.

Drought is a key *predisposition* driver and is one of the main environmental effects we are testing in our field trial. But pest and disease attack are also debilitating and may even be fatal. AOD is a good example of this. Both environmental and biological *predisposition* often act in concert and have amplified damaging effects than if they operate alone because the tree's energy sources are diminished, and defences are low.

Drought causes water deficiency and reduced growth in trees but nutrient imbalance also affects tree vigour and health. In the Bac-Stop project (Work Package 2) we ask the specific question: how do drought and nutrient stress affect AOD establishment and severity?

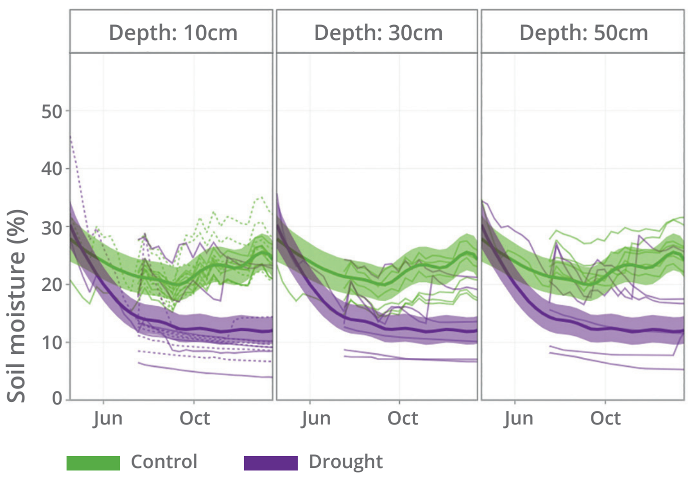

Figure 2: *Daily median soil moisture values recorded at three different depths (10cm, 30cm, and 50cm) in the control (green) and drought (purple) treated plots, between June and end of October 2021*

To answer the question, we have established a field trial on ~40-year-old Sessile Oak in which one third of the trees are subjected to drought stress which we are achieving by constructing rain exclusion shelters around the tree stems (See background Figure 1). Another third of the trees are almost fully ringbarked to induce nutrient and water deficiency stress, and the remaining third of the trees have been left as they are and serve as controls. Several months after the environmental treatments were applied, we inoculated the trees with the various components of AOD (more about this later).

The environmental conditions in the different treatments are monitored using soil moisture and temperature probes that are installed at different depths in the soils and linked to dataloggers. The dataloggers are battery powered, collecting hourly data that are downloaded at six weekly intervals.

So far, we can see that the drought shelters are working well. Using daily median values, we can see a significant difference between the drought and control treatments, with drought treatments having consistently lower soil moisture readings than controls (Figure 2). The difference in soil moisture levels between treatments has grown steadily over time but is starting to level off. This shows how the soils under the drought shelters are drying out, rapidly at first but slowing down with the passage of time, and may soon reach a very droughted state.

As the drought shelters extend only 2.5m on all sides of the trees, the roots of the affected trees have almost certainly sent out feeder roots beyond this distance in the search for water. This will have an energy cost and stress to the tree.

In terms of soil temperature, it was interesting that the drought treatment had consistently lower soil temperature during the summer months than the controls, and the drought shelters may contribute to this. Soil temperature decreases with depth and our data shows this clearly.

We expect the trees to respond to environmental stresses in measurable ways. For example, it is well known that drought stress causes changes to canopy temperature and leaf reflectance. When trees are placed under drought stress the leaf pores (stomata) close to reduce water loss, and leaf temperature goes up. When trees are stressed, the foliage may change colour for example, leaves may become lighter or turn yellow, and together with this there is a change in the reflectance of the leaves. Healthy leaves that are photosynthesising effectively are usually dark green in colour and have a low reflectance. So, leaves under nutrient and drought stress often have a higher temperature, are lighter in colour and have a higher reflectance.

To monitor and measure the effects of the environmental treatments on the trees, we have installed machines that are able to measure foliage reflectance, sap flow, tree girth, stem humidity, tree oscillation, air humidity and temperature in real time. The machines are called TreeTalkers (TT) and are strapped to the stems of trees (Figure 3). They have a number of probes, which are inserted into the stem, and multispectral sensors that measure all the above mentioned parameters. The data are transmitted every hour to battery powered wireless receivers (called TreeTalker Clouds), which are placed in the woodland and the data are stored in this '*Cloud*' and then downloaded remotely for analysis. These multimodal data tell us how trees are responding physiologically to the environmental treatments.

Figure 3: *A TreeTalker mounted on the stem of an Oak tree at the field trial*

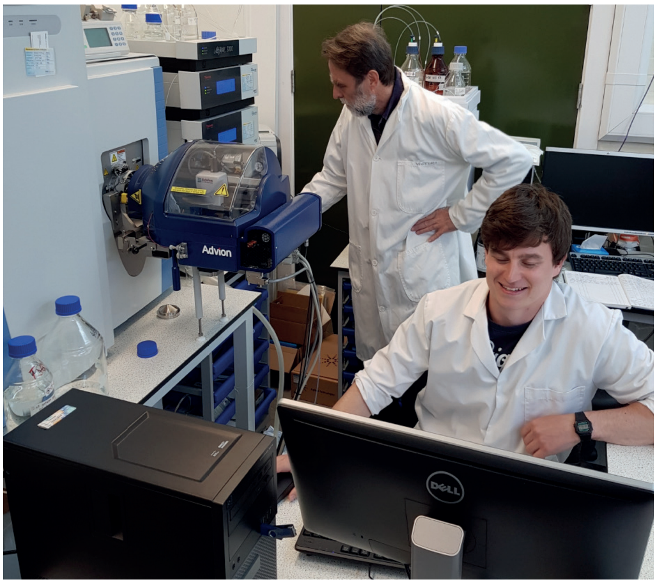

Figure 4: *Aberystwyth University team: Dr Jasen Finch (seated) and Prof John Draper in the laboratory, Dr Manfred Beckman (taking tree stem cores)*

So far, we can see a delayed response in tree growth in the drought treatments. It will be so exciting to see how this changes over the coming season, and whether we can detect changes in leaf reflectance.

At the beginning of the experiment, we measured the nutrient status of the soil, leaves and feeder roots by chemical analysis. This was repeated three to four months after the environmental treatments had been applied.

So far, the data suggest that droughted trees have smaller leaves, which will probably lead to a reduced photosynthetic capacity of these trees. In future analyses we hope to establish whether drought and ringbarking have also impacted the nutrient uptake of the trees. The inspiration and lead behind the TreeTalkers is Dr Elena Vanguelova.

We are also monitoring the impacts of the environmental effects on the tree's metabolism. In essence, the fundamental mechanisms of life are chemical interactions that take place within organisms. These interactions can change under different circumstances, for example in trees, when they become stressed or need to defend themselves.

Using chemical analyses, we are able to detect and pinpoint the changes that are taking place, which give us insights to the energy cost to the tree. Understanding this helps us work out how to assist trees to mitigate damaging effects. Dr Jasen Finch, Dr Manfred Beckman, and Prof. John Draper from Aberystwyth University (Figure 4) are carrying out this research and hopefully we will have some results on this in next year's edition of the Woodland Heritage Journal.

Once the environmental treatments had been applied to the trees and they had a few months to take effect, we applied the biological components that cause stem weeping in AOD. These treatments comprised a mixture of bacterial species and the larvae of *Agrilus biguttatus*, the Oak jewel beetle (also known as the two-spotted Oak buprestid - TSOB). We keep stocks of the bacteria in the laboratory at Alice Holt and so had only to grow these up to produce inoculum but getting the larvae of *Agrilus* was a much harder job.

To obtain larvae for experimental use we need to rear the beetles in captivity and so this is an annual event in our laboratory at Alice Holt, but it is reliant on a supply of beetle infested wood which we ask from woodland owners and managers. Because *Agrilus* has a two-year lifecycle that takes place underneath the bark and hence out of sight, we can never be sure if the bark slabs we get from our supporters have a lot, or a few, or even any (!) pupating beetles that will emerge in the insect emergence cages we have constructed at Alice Holt. Obtaining sufficient beetles is definitely the most nerve-racking part of the work, and every year we have this worry!

From December to April, we visit sites and ask people to donate slabs of wood from trees we believe to be infested with *Agrilus* larvae. We place these wood slabs inside large beetle emergence cages and in the late spring or early summer the beetles emerge from the bark into the cages, where we catch them and take them to the laboratory for analysis and beetle rearing.

To rear beetles, firstly we examine and determine the gender of the beetles and then place small numbers of males and

Figure 5: *Dr Michael Crampton preparing bacterial inoculum in our new mobile laboratory*

Figure 6: *A plastic PCR tube fixed to the stem of the tree ready to receive bacterial inoculum*

females together in small breeding cages that contain fresh Oak leaves, water and a sugar solution. There, beetles feed until mature and then mate. The females lay their eggs either on the blue paper towel at the bottom of the cage or in folded blotting paper, which is intended to mimic bark cracks. The egg clusters are cut out from the paper and placed in Petri dishes in incubators. To try and co-ordinate a hatching period we incubate eggs at various temperatures, the cooler it is the longer it takes for the larvae to hatch from the eggs.

The past year we had a rather low number of beetles relative to the number we required for all our work, but we did manage to get a sufficient number of eggs and hatching larvae to inoculate the trees. Michael Crampton carried out the beetle rearing for us over the year gone by but has now left our team for promotion in another team; we will really miss the excellent work Michael did for us.

Having beetle eggs and bacterial cultures in hand we headed to the field in our new mobile laboratory (Figure 5). This is a Sprinter van that has been specially fitted with stainless steel benching, electricity, incubators, and cupboards. Now we can take microscopes, centrifuges, and other laboratory equipment safely into the field, and this enables us to prepare accurate amounts of inoculum and to work aseptically. What a huge improvement and boost for us! I remember the 'old days' when we did this type of work in a much more 'Heath Robinson' style.

One of the challenges we faced was how to apply liquid inoculum to standing trees in a safe way and so that it would not just run down the side of the tree trunk and be lost. Dr Anparasy Kajamuhan (Anbu) and I used tiny plastic tubes with the tips cut away at an angle that we secured to tree trunks with silicon ear plugs (Figure 6). *The joke in the team was that it had not escaped me that we were using ear plugs for our work at Little Snoring!*

Moving on ...! Michael and Sunny (Sundeep Kaur) prepared the bacterial inoculum in the mobile laboratory while Anbu and I applied the tubes to the tree stems. In instances where we applied inoculum by wounding the tree first, we made a small wound with a cork borer and then inserted the bottomless tubes in the wound. We then used a pipette to add inoculum to the tubes and left them overnight so that the inoculum could soak into the tree. The following day we removed the tubes where necessary and gently applied the beetle eggs and larvae. A video demonstrating this work is available on our BPD (Bacterial Plant Diseases) website. **bacterialplantdiseases.uk/bac-stop-research-in-action**

We hope to obtain sufficient beetles this year to repeat the inoculations and mimic nature, where the beetles would be expected to repeat their egg laying on trees until the tree was no longer suitable as a safe-haven and nutrient source for the developing larvae. Final measurements of the effects of all these treatments will be made in the autumn of 2023 and results should be ready the following summer.

The final aspect of our work examines the effect of the environment and biological stresses on the tree *microbiome*. A *microbiome* is the total community of microbes living in and on an organism. We all have unique *microbiomes,* for example human microbiomes are quite different from plant *microbiomes* and each *microbiome* reflects how organisms function. Thus *microbiomes* are important because they are part of the way in which organisms live. For example they aid nutrient acquisition and digestion, offer protection, and defend organisms against attack and ingress. Their composition is linked to their function and environmental conditions, and under environmental stresses the natural healthy balance of the *microbiome* may be tipped with deleterious consequences for the tree.

Professor James McDonald's group at Bangor University is investigating this aspect of the trial, where Usman Hussain is doing his PhD on the microbiomes of the trees in the trial. Lucy Corbett at Reading University is doing her PhD on the enzymes in the soils around the feed-roots under the guidance of Prof Liz Shaw.

The key questions we hope this research will answer are:

- How do trees respond to environmental stresses?
- What effects do these stresses have on tree physiology, nutrient status, and resilience?
- Is *Agrilus* essential to the development of AOD and if so, what mechanisms are responsible for this?
- Is wounding necessary for AOD to develop?
- How are the biology of trees and their microbial communities impacted by drought and nutrient stress?
- What consequences do drought and nutrient stress have on tree health and resilience?

All the research we are carrying out aims to improve management. However, the road to evidence-based guidance is long. In the interests of space, I will have to report next year on the work we are doing to find out if *Agrilus* carries and transfers the bacteria causing stem lesions. These are essential questions that require answering if we are to consider beetle management.

However, I want to change tack a little now and share some concerns I have about management of Oak trees with Chronic Oak Decline (COD), particularly those Oak in parkland settings and heritage gardens, which include ancient and veteran Oak trees.

Managing Chronic Oak Decline (COD)

Chronic Oak Decline is also a *Decline syndrome* of Oak. It is different to AOD in that there is no bacterial stem bleeding component, although stem weeping caused by other damaging agents may occur. It is not as well defined as AOD, and the symptoms are not very well characterised or linked specifically with causal agents. This is partly because typical symptoms may be caused by many agents acting alone or together. There is an urgent need for research to provide clarity on the symptoms, causes and management of this Decline syndrome. In old currency, COD aligns with Oak dieback.

Our current thinking is that COD is driven primarily by environmental predisposition and root problems. Damage to the buttress roots and tree collar, the area of the tree at the soil surface interface, are thought to be a key feature of the disease syndrome and may be brought about by fungal pathogens or mechanical damage. Diminished feeder roots and impaired function are also thought to be important in the syndrome.

Soil health, in terms of soil structure, physical properties, balanced soil chemistry, and microbial function, is the cradle of tree health and vitality. The connection between Decline diseases, tree root health and mycorrhizal fungi is considered fundamental to tree health, and so there is real concern that damage to, and impoverishment of the root systems will translate to a significant predisposition driver for Decline diseases.

Identification of trees with COD relies on the condition of the tree crown but may also involve destructive sampling of buttress roots and the tree collar. Additionally, observations on fungal fruiting bodies (mushrooms, stem brackets or rhizomorphs, which are commonly known as boot laces) are extremely valuable in providing clues to primary biotic drivers. The symptoms of the disease also take quite a long time to develop, and so monitoring records of changes in the tree's physical appearance are also very helpful in diagnosis.

Typically, Oak trees suffering from COD initially show thinning of the crown and foliage may turn a lighter colour, perhaps even become yellowish (called chlorotic). This may occur over the whole crown or just over different segments of the crown. The tree sheds fine twigs and as this happens the branch ends appear to be stubby. Epicormic shoots may develop along the branches or stems. Major branches begin to dieback, there is no secondary crown below the branches that are dying back. (Note: if there is a secondary crown in good condition below the branch dieback, then the trees have a different condition called "stag-headedness". Stag-headedness usually occurs when tree buds are swelling or just flushed and a very hard frost kills the branches, leading to a temporary dieback and crown retrenchment. If tree health remains good the frost-killed branches will fall off and tree growth will resume. This may take years!). Destructive sampling of the buttress roots and collar may reveal attack by damaging root pathogens.

I was dismayed to see the very poor condition of mature Oak in such settings when I visited several sites this year.

Figure 7: *Aerial photograph reveals the extent of dieback and death of Oak trees due to Chronic Oak Decline*

Figure 8: *Turkey Oak, more than 400 years old, now suffering from an* Armillaria *incursion. Will it survive?*

At one site, Oak reaching their prime were showing alarming symptoms of dieback as shown in the aerial photo taken in 2020 (Figure 7). By trawling back through scattered monitoring records we were able to suggest that the death rate of Oak in their prime at this site had doubled in the past 10-15 years and that of Sweet Chestnut had increased ten-fold.

At another site, a beautiful turkey Oak (Figure 8) more than 400 years old was being attacked by *Armillaria*. A key concern and question arising from this alarming situation is how are we managing today's trees for tomorrow's ancient and champion trees? There is an urgent need for a conversation about a national tree management strategy to ensure trees reach full maturity and ancient status.

It is fair to say that because our research focus has been centred on AOD perhaps I have not given the attention needed to trees' suffering from COD, but I have tried to progress relevant research on the topic. We have a student at Bangor University, Bethany Pettifor, who is carrying out some foundational work on the Oak buttress root pathogen *Gymnopus* (previously known as *Collybia*) and next year we should report on some of this work. I also have small projects on *Armillaria* and hopes to develop this area in the future. So, I am making an effort now to give this serious condition, and management of it, some attention.

I am really grateful for the support from landowners and managers, and for their incredibly generous attention and help. The National Trust and Natural England are extremely keen to get us involved and they have been very helpful in both AOD and COD research projects; I thank them very much.

We hope to involve as many people as possible in considering the best management of these national arborical icons, and offer some evidence-based guidelines to ensure the good health and resilience of our trees for future generations in the face of changing climates and disturbed environments.

Monitoring the health of our Oak trees

Nathan Brown

The health of trees is reflected in their crowns. The number of leaves they have on their branches influences the amount of energy they can make through photosynthesis. By monitoring crown condition, we gain an insight into the underlying heath status of Oak, which in turn can help reveal long-term trends and the potential impact of environmental factors that may act to predispose trees to decline.

Oak crown condition is monitored by Forest Research at eighty-five plots across Great Britain. These plots provide vital historical context. They were established in 1989 when concerns about pollutant deposition and acid rain were prominent but it is unclear how representative they are of the nation's Oaks more generally. All plots are in Oak dominated woodland and this means that trees outside woodland are not well represented at all.

Trees in the wider treescape are likely to be subject to several additional factors which may impact their health, for example: soil compaction from humans and animals; the impacts of agriculture through ploughing and the application of fertiliser as well as exposure to higher temperatures and wind speeds. The Bac-Stop project is currently exploring the potential for volunteer groups to monitor their local trees and provide vital information to address existing gaps in Oak condition monitoring.

The first year of the project has focused on method development. The initial workshops were online and relied on images of trees from Google Street View to underpin the training. However, in September 2021 we could finally emerge into the field, hold in-person training events, and see the trees themselves! A big thank you to all the volunteers who gave their time to help our project!

We are currently in a strong position for 2022: we have a reliable method, training materials and recording systems, and now we are ready to expand the scope of the project. We hope to work with groups at five sites in the coming year and we plan to use a mixture of all the methods trailed so far.

We will ask volunteers to attend a one-hour webinar, which will be followed by a half-day training workshop in the field. This should provide all the information and tools for further monitoring, enable individuals to observe the trees that are most significant to them, and provide data that can be integrated with an established monitoring programme. One of our key aims is to instil an interest in observing change in the natural environment, as well as generating some useful data for research.

Investigating land-managers' and the public's attitudes to Oak and Acute Oak Decline and its management

Liz O'Brien

Our work in Bac-Stop focuses on people – including landowners and managers, and the public. We have conducted seventeen interviews with landowners and managers of Oak so far to explore the ways in which they value Oak, and the different types of management actions they already or would undertake to conserve and protect their Oak trees and woodlands. We cover issues such as their current management actions and awareness of tree pests and diseases, and any surveillance and monitoring they undertake, as well as their perceptions of risk from pests and diseases. Our interviews include different types of land managers from those who manage Oak or mixed woodlands to those who manage parkland estates, and include managers focused on conservation, timber production, and multiple objectives.

We have also conducted a survey of 6,000 UK residents to explore people's connection to nature, how people feel about Oak, and to what extent they value Oaks in different settings from woodlands to hedgerows, for example. We also explored people's knowledge of tree pests and diseases including Acute Oak Decline, and their views on the acceptability of different management methods to protect Oak.

We have also asked about people's willingness to get involved in volunteering activities to monitor or help to manage AOD. We asked the public when thinking of Oaks what adjectives and emotions come to mind. The word cloud below illustrates the range of words people used; the larger words mean more people used those words to describe how they feel about Oaks.

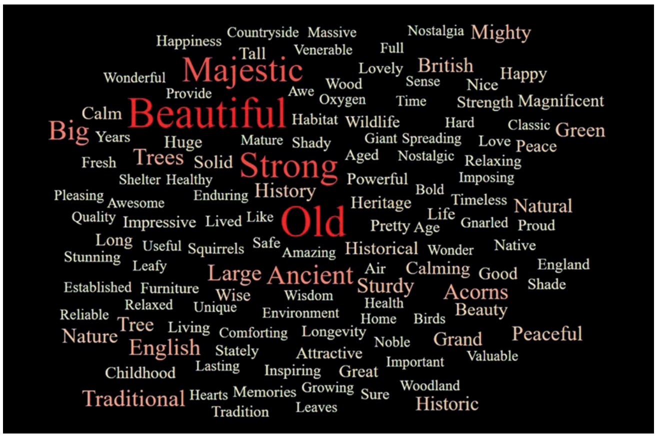

Figure 9: *A word cloud capturing the range of words people frequently used when asked how they feel about Oak trees*

We are also capturing data through our 'Odes2Oak' campaign where we are asking people to pay a tribute, celebrate or to outline how they feel about Oaks. One making a tribute provided this photograph and the following caption:

Figure 10: *Favourite Oak from an entry to the Odes2Oak website where people are invited to let us know how they feel about Oak trees*

"Working in a special needs school the children walk past many beautiful Oak trees in our beautiful grounds..... this is a favourite and you can see our other favourite far in the background."

Please would you help? We are asking for photographs or poems, artwork and written experiences concerning the importance of Oaks to people whether it is an individual tree, Oak products, or Oak woodlands. There is still a chance to submit a contribution. We would love to hear from you. Please go to: **bacterialplantdiseases.uk/odes2oaks**

We are currently analysing our survey, interview, and Odes2Oak data which will highlight whether different sections of society value Oaks for different reasons and identify any variations in perceptions between land managers and the public concerning the acceptability of the various methods that can be employed to protect against Acute Oak Decline. We will be reporting our results in 2022.

An update on AOD research activities at UWE (University of the West of England, Bristol)

Carrie Brady

In my update for the last Journal, I wrote about our *Tilia* (Lime) tree sampling project where the bacterial community of healthy trees is compared to those showing bleeding symptoms. A research Master's student, Helene Kile, joined our group at UWE in March this year and started by screening the material (swabs, leaves, twigs) I had collected from *Tilia* hybrids at Westonbirt Arboretum in September 2020.

A single *Tilia* × *moltkei* tree was displaying bleeding symptoms during that visit, which was swabbed. The molecular identification of the bacteria cultured from the swabs revealed the presence of a new *Brenneria* species found only in the diseased material. This was an exciting discovery as *Brenneria* is a genus of bacteria known to cause cankers, necroses and wilts on a range of woody hosts. The other plant material sampled from both symptomatic and healthy *Tilia* yielded typical endophytic bacteria such as *Erwinia billingiae*, *Pseudomonas* sp. and *Pantoea* sp.

We returned to Westonbirt in June 2021 to re-sample the same *Tilia* hybrids. In the week preceding our visit, the symptomatic *Tilia* tree was uprooted and completely blown over during a storm. Although the loss of a majestic tree was unfortunate, it did allow Helene and I to sample extensively and invasively.

Figure 11: *Necrotic lesion underlying the bleeding outer bark of a Tilia × moltkei, at Westonbirt*

Figure 12: *Helene Kile sampling an actively bleeding Tilia × europaea, at Minchinhamptom*

We found several more bleeds higher up the trunk of the tree which were swabbed. The outer bark panels containing these bleeds were then removed, revealing shallow, water-soaked, black necrotic lesions extending longitudinally down the trunk. The largest of these was 110 cm in length (Figure 11). The new *Brenneria* species was again isolated only from the diseased material, suggesting this bacterium plays a role in the necrosis.

Over the course of the summer, additional symptomatic *Tilia* hybrids were sampled from sites in Gloucestershire and Wiltshire with bacteria cultured from the diseased material identified as belonging to the new *Brenneria* species (Figure 12). Additionally, isolates of *Brenneria goodwinii* and *Gibbsiella quercinecans*, the two bacteria responsible for the necroses observed in AOD symptomatic Oak, were also cultured from necrotic lesions at some sites.

Helene has worked diligently in the lab performing all the analyses necessary to describe a novel bacterial species and is in the process of writing the publication after successfully defending her dissertation.

With further cases of bleeding cankers observed on *Tilia* in North Somerset last month, interesting questions have been raised regarding the possibility of another polymicrobial disease on a broadleaf host, similar to the complex responsible for AOD.