Protecting our Woodlands: tackling tree pests and diseases

16th - 17th September 2013
University of Reading
Welcome to the BSPP Protecting our Woodlands meeting. The increase in the level of interest in tree diseases in the media recently prompted the BSPP to organise this meeting and judging from the level of interest from the scientific community, it is important to researchers as well. This meeting showcases some of the current work relating to tree diseases from many scientific disciplines.

I would like to thank the people who have helped organise this meeting; Steve Whisson, Steve Woodward, Rob Jackson, Murray Grant and Joan Webber and Julie Ellwood for her administrative support. I would also like to thank Optigene for supporting this meeting.

I hope you find this meeting both informative and enjoyable.

Elizabeth Orton
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Alien Invasive Pathogens: The Major Threat to Forest Ecosystems in a Time of Rapid Climate Change

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Forest ecosystems face unprecedented challenges from the rising rate of influx of alien invasive pests and pathogens resulting from increased global trade. Moreover, climate change predictions suggest that many additional alien pests and pathogens may become problems in forests as temperatures increase and rainfall patterns change. Recent indications of the potential for pathogens to transfer between hosts and the inter-specific hybridisation possible between related pathogens increase concerns over the possibility of escalations in damage in the future.

Using examples drawn from wide-scale environmental damage caused to tree populations and forest ecosystems in Europe and elsewhere in the world we will illustrate the potential of alien pathogens to alter ecosystems drastically and to impact on human requirements of forests. Classic examples of devastation by alien invasive pathogens affecting trees include *Cryphonectria parasitica* wiping out American chestnuts, *Ophiostoma novo-ulmi* killing elms, Jarrah dieback caused by *Phytophthora cinnamomi* and white pine blister rust arising from trans-Atlantic transfer of *Cronartium ribicola*. Increasing numbers of damaging invasive pathogens have become established in Europe in the past 25 years.

Reasons for the alien invasive pathogen problem hitting the headlines in recent years will be discussed: Why is this problem increasing so rapidly? What are the root causes of the problems? Is there anything that can be done to reduce the influx of alien invasive pests and pathogens, or to reduce the damage caused once they become established?
Species of the genus *Phytophthora* are prominent among the recently emerging pathogens that threaten our trees. The study of these fungus-like organisms has been relatively neglected, partly due to their intractability for genetic manipulation. Furthermore, little is known about genetic diversity within populations of these pathogens within the UK. We have taken advantage of low-cost high-throughput genome sequencing to make inferences about the biology of *P. kernoviae, P. lateralis* and *P. ramorum* (lineages EU1 and EU2), especially focusing on the repertoires of genes potentially encoding virulence effector proteins. By sequencing multiple isolates of each species, we were also able to detect hitherto undiscovered genetic variation, some of which could be exploited as molecular markers for epidemiological tracking of pathogen spread.
Molecular tracking of *Phytophthora* threats in natural ecosystems

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A failure to detect the ‘unknown’ is a recognized weakness of protocols for international plant health legislation. *Phytophthora* species pose serious challenges to tree health on a global scale and yet more than half of the described species were unknown to science 15 years ago. The development of sampling methods and a genus-specific molecular detection system has provided us an opportunity to discover and track these pathogens. Results of the on-site use of a knapsack sprayer and in-line water filters as an effective means of sampling *Phytophthora* propagules from streams will be presented. rDNA-based detection and barcoding of the samples revealed a surprisingly high diversity of species from streams flowing through a range of apparently ‘healthy’ Scottish ecosystems over the course of a year. With ever-reducing costs of high throughput sequencing systems, advances that would allow routine detection of existing pathogen species and potential new threats to forests and support plant health legislation will be discussed.
Study of the lineages of *Phytophthora ramorum* in Ireland

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*Phytophthora ramorum* has been reported affecting a wide range of hosts during the last two decades. It was first reported in the UK in 2003 on Rhododendron and in 2009 on Japanese larch. Since then over 500ha of larch have been felled in Northern Ireland alone. *P. ramorum* has four lineages: NA1 and NA2 found in North America and EU1 and EU2 only found in Europe. The fourth lineage, EU2, was only identified in 2012 and to date has only been reported in the N. Ireland population and from a small area in the South-West of Scotland. The lineage of over 300 isolates from N. Ireland and the Republic of Ireland has been determined using RFLP and microsatellite markers. There is a majority of EU2 in NI (89%), with rare EU1 distributed in located areas that have been shown to have probably been introduced come from previously infected nurseries. All of the RIO isolates are EU1. The data are being further analysed using Arlequin 3.5. to identify evidence of grouping of isolates. Preliminary results of the generic variation obtained from the microsatellite markers show that the Northern Irish population is very similar (96%) which suggests that all the outbreaks may have come from the same introduction.
Invited Speaker
Responses to new tree pathogens; *Pseudomonas syringae* pv. *aesculi* on horse chestnut and *Phytophthora austrocedrae* on juniper

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Information-gathering on new tree diseases requires both epidemiological and genetic approaches in order to understand pathways of introduction and spread. *Pseudomonas syringae* pv. *aesculi* (*Pae*), which causes bleeding canker of horse chestnut, is a pathogenic bacterium believed to have originated in India. The pathogen can enter branches aerially, *via* lenticels, leaf scars and nodes, causing lesions in the cortex and phloem. Cankers expand, leading to bleeding symptoms and progressive crown dieback. Genomic data suggest that *Pae* probably spread to Europe from India via an unknown pathway, with the epidemic in Britain resulting from the introduction of a single bacterial strain. Genomic comparisons with 27 other *P. syringae* pathovars showed that *Pae* has readily gained and lost hundreds of genes during its recent past. The genomes of a further 26 *P. syringae* pathovars from woody hosts are currently being analysed to elucidate genetic and genomic differences underlying niche adaptation. Another pathogen, *Phytophthora austrocedrae*, which was first described in 2007 associated with widespread dieback and mortality of *Austrocedrus chilensis* in Argentina, was not known to occur anywhere else in the world until 2011 when it was found killing juniper in northern Britain, predominantly through root infections on wet sites. Genetic analyses so far suggest that the pathogen is probably an introduction to Britain, but is unlikely to have come from Argentina. Studies are currently focused on understanding the life cycle and disease epidemiology of *P. austrocedrae*, its pattern of distribution on juniper, and its capacity for spread aerially and in soil.
Diagnostic approaches to tackle identification of *Chalara* ash dieback and other forestry pathogens.

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Recent findings of a number of pathogens threatening our native tree species have highlighted the requirements for rapid, sensitive, fit for purpose and accurate diagnostic approaches to identify causal agents of disease. *Chalara* ash dieback (*Hymenoscyphus pseudoalbidus*) pathogen is a serious threat to the UK’s native ash tree (*Fraxinus excelsior*) population. It was first detected at a nursery in February 2012 on ash saplings, followed by a woodland finding in late October on more mature trees. *Phytophthora ramorum* and *P. kernoviae* affect a range of tree and plant species. Most recently *P. ramorum* has been identified killing vast numbers of larch (*Larix kaempferi*) in the west of the UK.

Diagnostics of these diseases begins in the field with identification of symptoms. As these can be highly variable, a confirmatory field or laboratory based approach is usually necessary. Following the first wild finding of ash dieback, ministers called an emergency COBRA committee meeting to tackle the problem. A national survey was commissioned to identify the extent of the disease, and was required to be completed within 5 days. This unprecedented diagnostic requirement was achieved by identifying the most appropriate approach to tackle this requirement in the laboratory and implementing various strategies to meet this goal.

Identifying the presence of pathogenic organisms in the environment without visible host symptoms may give an indication of new areas that could become infected. Diagnostic tools to aid this involve various trapping techniques that can be used in different situations, depending on the pathogens nature.
Real-time response to invading plant pathogens: modelling Chalara dieback of ash


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When an invading pathogen is detected, policy-makers are presented with a number of questions. How did it get here? Is it still coming in? Where might it be already? Where will it spread next? What can be done to stop it? How much would this cost? These questions must necessarily be answered quickly, when epidemiological data is scant.

We illustrate this using modelling work targeting ash dieback, caused by Chalara fraxinea. The pathogen was first detected in the U.K. in 2012, although it has been spreading across continental Europe since the early 1990s. The atmospheric dispersal model NAME was used to show that U.K. “wider environment” infections could be the result of wind-borne inoculum from the continent. A landscape-scale epidemiological model was then used to predict risks of future within-U.K. spread. This model tracks the pathogen on an accurately resolved ash host landscape at 250m x 250m resolution, and was parameterised by fitting to the accelerating wave of infection that has spread across continental Europe. Within the next ten years, 90% of the ~200,000ha of ash in the U.K. is predicted to become infected. Large-scale mitigation is therefore unrealistic. However, the model indicates that control intervention, for example removing infected recent plantings, can slow the spread to currently uninfected regions, in some cases significantly.

Our results were used to inform U.K. government policy, and directly appear in DEFRA’s Chalara Management Plan. They show how a mathematical model can used in real-time to advise governmental response to an invading plant pathogen.
Loop-mediated isothermal amplification for detection of tree pests and diseases in the field

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Nucleic acid-based testing allows rapid and accurate detection of plant pests and pathogens in the laboratory, and recent developments making these methods faster and simpler have opened up the possibility for testing to be carried out in non-laboratory conditions. The ability to perform testing in the field eliminates the delay between sampling and obtaining a result, and this has the potential to bring significant benefits in outbreak situations. One technology that has demonstrable advantages for testing in non-laboratory conditions is loop-mediated isothermal amplification (LAMP), which can be used in conjunction with extremely simple sample processing and an easy-to-use, battery powered instrument to obtain results in 30-40 minutes. A LAMP-based assay has been developed for detection of *Chalara fraxinea* in symptomatic ash wood. The assay has been incorporated into a simplified workflow suitable for use in the field, and validated in line with the European and Mediterranean Plant Protection Organization (EPPO) standard, and by comparative testing of naturally infected samples in the laboratory and in the field.
Invited Speaker
The epidemic and biological control of chestnut blight in Europe

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The chestnut blight fungus *Cryphonectria parasitica* is native to East Asia and has been introduced into North America and Europe. In both continents the pathogen caused a severe disease epidemic on the susceptible American (*Castanea dentata*) and European (*C. sativa*) chestnut species. In Europe, however, many chestnut stands recovered from the disease due to the spontaneous occurrence of hypovirulence. Hypovirulence is caused by *Cryphonectria hypovirus 1* (CHV-1) that infects *C. parasitica* and reduces its virulence and sporulation capacity. Natural dissemination and active biological control applications have lead to a high prevalence of the hypovirus in Europe. Where natural hypovirulence is widespread, the disease incidence is still high, but disease severity is usually low. Factors found to contribute to the success of hypovirulence include (1) efficient dissemination of hypovirus-infected propagules; (2) low vegetative incompatibility barriers for virus transmission between fungal individuals; (3) presence of dead chestnut wood that supports the production of hypovirulent inoculum; (4) ecological fitness of the main biological control agent, CHV-1 subtype I; and (5) lower susceptibility of European compared to American chestnut. Whether the biological control system is sustainable remains to be seen. There is concern that the diversity of vegetative compatibility types could increase in Europe through sexual reproduction between *C. parasitica* genotypes originating from different founder populations. A higher level of vegetative incompatibility would not only hamper hypovirus spread within a population but could also select for lower virulence in CHV-1 and subsequently lead to an erosion of biological control.
A potential role for bacteriophages in mediating tree disease?

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The dynamic microbial communities of long-lived hosts will change over time due to immigration of new species, interaction with the host defence system, and selection by bacteriophage viruses (phages), but the relative roles of each process are unclear. To determine whether phages in the natural environment influence the bacterial communities living with the leaves of trees, I have been following communities of bacteria and phages living within the horse chestnut tree. I have demonstrated both that phages are well adapted to their local bacterial hosts and that these phages are selecting for resistant bacterial strains over time. These local adaptations and reciprocal changes suggest that phages indeed play a key role in shaping the microbiota of tree leaves, and therefore that they may influence whether or not a given individual is susceptible to infection by bacterial pathogens. I will discuss the results in light of what we currently know about the influence of phages in shaping bacterial virulence and also in changing the density of susceptible bacterial host populations. I will also briefly examine the potential role for phages as a biocontrol tool in treating disease.
Dieback and decline on pedunculate (Quercus robur) and sessile (Q. petraea) oak is recognised as a complex syndrome. However, discerning evaluation of the disorder in Britain revealed a distinctive condition called Acute Oak Decline (AOD), within the broader concept of oak decline. AOD is identified by weeping patches emanating from fissures between bark plates on trunks of established trees. Beneath the outer bark extensive necrosis of the inner bark occurs frequently resulting in the formation of cavities. Larval galleries of the oak Buprestid, Agrilus biguttatus, were also present on the cambial-phloem interface. It was hypothesised that tissue necrosis was caused by biotic factors.

A study using conventional isolation techniques to determine putative biotic causal agents was carried out on 21 symptomatic and 9 healthy trees from 17 sites across England. A range of selective media was used to plate pieces of surface disinfested tissue. Various fungal species were isolated but at low incidence and frequency. By contrast, bacteria were isolated with high incidence. Most bacterial taxa were novel species. The bacterial species composition of healthy trees was significantly different to symptomatic ones (P=0.001). Healthy trees were characterised Gram-positive bacteria whereas symptomatic trees had high levels of Gram-negative bacteria. Brenneria goodwinii and Gibbsiella quercinecans, were consistently isolated from symptomatic tissue but not healthy trees, multiple Pseudomonas spp. were present in both healthy and symptomatic trees. Results lead to the hypothesis that Brenneria goodwinii and Gibbsiella quercinecans play a role in tissue necrosis in AOD.
Phytophthora species in water courses in Scotland

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Phytophthora species are known as destructive pathogens in agriculture, horticulture and forests. Recent years have seen the uprising of Phytophthora ramorum, P. kernoviae, P. lateralis and P. austrocedrae in the UK, all being major threats for various parts of British woodland and other environments. Plant Health legislation tries to prevent introduction, establishment and further spread of these pathogens.

However, Phytophthora species are also a normal part of the natural environment. They have been found in forest soils and streams, often without causing any obvious disease symptoms and their role is still poorly understood. Commercial plant growing is often closely monitored for diseases by the growers and official Plant Health authorities whereas surveys of the environment are rare and much more challenging.

In the presented work a baiting method with rhododendron leaves was used to survey 66 river sites in North-East, Central, West and South-West Scotland between October 2012 and May 2013. The baits were left in the streams 2-6 days before they were re-collected and plated on Phytophthora-selective agar. Species were identified initially by sequencing of the ITS region and then if required by further sequencing of the COXI, COXII and/or beta-tubulin gene.

This work is meant as a first step to produce a record of Phytophthora species established in the Scottish environment. Such records would help to assess the risk associated with those species and to avoid unnecessary quarantine measures against species already established in the environment.
Invited Speaker
Ash dieback an emerging disease and *Heterobasidion annosum* an old pathogen of conifers in Europe.

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Since the mid 1990’s, common ash has been attacked by a new disease in Europe, and is today covering most of the ash distribution except for southwestern parts of Europe. It took about ten years before its causal agent, the ascomycete fungus, *Hymenoscyphus pseudoalbidus*, was described. Based on the genetic variation of the species, we now know that the fungus originates in eastern Asia. *Fraxinus excelsior* is a highly susceptible host, something that is reflected in the very low proportion of resistant trees in the population. The life cycle of the fungus includes infection of leaves in the summer that eventually spreads into twigs, branches and stems, causing severe dieback and eventually death of the whole tree. The fungus survives saprotrophically on leaf petiols that become sclerotized. Host pathogen interaction involves toxic compounds e.g. viridiol that the fungus produces. Recent work on characterizing pathogenicity of the fungus has involved sequencing its whole genome and for comparison also its close saprotrophic relative *H. albidus*. By contrast, *Heterobasidion annosum* s.l. is an old disease causing root rot to conifers. It is favoured by forestry and today well established in plantations and natural forests. Characterization of host pathogen interaction has been widely helped by whole genome sequencing and genome wide association studies in the fungus.
Epidemic modelling and surveillance strategies for invading tree pests and pathogens

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Effective disease control depends upon early detection of invading epidemics. Early detection relies on the deployment of limited sampling resources across a landscape. However, how the deployment of sampling resources relates to the probability to detect an invading epidemic is not well understood. As a result, large scale sampling programs are often ad-hoc and thus sub-optimal. We demonstrate an epidemiological modelling method that can determine the incidence an epidemic will have reached when it is discovered for the first time based on a surveillance program that takes regular samples from a host population. The general method reveals that incidence-at-first-discovery can be calculated from a simple function relating the epidemic growth rate and the frequency and intensity of sampling. We show how this can be tailored and validated for specific pathogen problems. Additionally, using a stochastic optimisation approach we show how sample placement across a landscape can be optimised to maximise the probability to detect an epidemic. We find that, when the sampling objective is early detection, the optimal course of action is usually to spread sampling resources evenly in the landscape, even when disease risk is clustered. However, following the first discovery of an epidemic the sampling objective may change and become one of searching and finding as many new outbreaks as possible. In which case spatially targeting high-risk clusters is the optimal course of action. The methods have been applied to both P. ramorum and Chalara in the UK.
Biotechnology and Nigeria's Forests Health

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In Nigeria, Africa’s most populous nation (167 million), the loss of forests is occurring at an alarming rate, a consequence of increasing population pressure, pests and diseases. The nation's southern rain forest, the source of the country’s timber resources, now covers only 2% of the total land area. FAO warns that the nation's remaining forest area will disappear by year 2020 if the current 3.5% yearly depletion rate is maintained. There is need to address the significant forest loss due to pest and diseases through biotechnology, in order to ensure sustainable provision of healthy forests and forest products including wood. The development of healthy forests and forest products takes time no doubt, but biotechnology helps to shrink the time. This paper reviews the state of biotechnological activities aimed at protecting Nigeria’s forests against pests and diseases.
OPAL (Open Air Laboratories) Tree Health Survey and other citizen science activities to encourage engagement in plant health

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The recent incursion of ash dieback (*Hymenoscyphus pseudo-albidus*) has clearly demonstrated the public’s concern about non-native plant pests and pathogens and an appetite to understand more about these problems. The UK Plant Health Service carries out statutory work to prevent their introduction by inspection of plants and plant products at the point of entry and monitoring for their presence within the UK. This involves checking of documentation, physical examination and laboratory testing, checking of documentation and working closely with stakeholders to raise awareness about harmful pests and pathogens. This has been supported by new initiatives such as the ‘Tree Health and Plant Biosecurity Expert Task Force’ and the ‘Chalara Management Control Plan’ to further strengthen biosecurity and reduce the rate of spread of pests and pathogens. This paper will discuss a number of ongoing and new initiatives developed by the UK Plant Health Service to encourage engagement from stakeholders, industry and members of the public with the latter concept sometimes referred to as ‘citizen science’. A recent example of such initiatives include the OPAL (Open Air Laboratories) Tree Health Survey which is the seventh national OPAL survey, launched in May 2013, with the aim to help people to learn more about trees, how to assess their general health and how to monitor for tree pests and diseases and report potential findings of the six ‘most unwanted’. This paper will illustrate aspects of the survey and provide feedback on progress to date. It will also elaborate further on similar activities such as the recent RHS ‘Stop the Spread’ Show Garden in addition to new national and international projects concerning tree health.
Epidemiology of Acute Oak Decline in Great Britain

Nathan Brown\textsuperscript{1, 2}, Sandra Denman\textsuperscript{2}, Xiangming Xu\textsuperscript{3} and Mike Jeger\textsuperscript{1}

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Oak has long been affected by decline, with host vigour reduced by many interacting causes. Oak decline is traditionally described as chronic, a slow process occurring over decades. In recent years a rapid form of decline has been observed in England, affecting both native species \textit{Quercus petraea} and \textit{Q. robur} and termed Acute Oak Decline. Investigations have identified a key bacterial component, involving several new or re-assigned taxa. Distinctive symptoms are visible on the trunk in the form of dark exudates (“bleeding cankers”) that flow between bark plates. Below the surface these are often (>90\%) associated with larval galleries in the inner bark. A four-year study on the spatial and temporal dynamics of bleeding cankers and occurrence of \textit{A. biguttatus} was completed in 2013. Eight geographically separated sites in England were established in order to: examine the correlation of stem symptoms with tree health and mortality; examine the within site pattern/s of spread to new hosts; and examine the link between stem bleeds and exit holes. At each site a complete mapping was made in which the status of all oak trees (n=115-260 trees/ per site) was assessed in terms of: i) symptom (stem bleed) presence and absence; ii) presence and absence of exit holes of \textit{A. biguttatus}; iii) co-occurrence (bleeds and exit holes); iv) and overall tree condition, including mortality. A modified version of the well-known Ripley’s k-function, was used to characterise clustering at each. These analyses describe the spatial patterns at each site and their annual development.
The Utility of the Intergenic Transcribed Spacer Region 1 as a Molecular Marker for the Identification and Discrimination of Enterobacteriaceae Associated with Acute Oak Decline (AOD)

James Doonan\textsuperscript{1}, Sandra Denman\textsuperscript{2}, Christoph Gertler\textsuperscript{1}, Justin A Pachebat\textsuperscript{3} and Peter Golyshin\textsuperscript{1} and James E McDonald\textsuperscript{1*}

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The molecular identification of environmental Enterobacteriaceae strains from trees affected by Acute Oak Decline (AOD) is a laborious and costly process, particularly when a large number of isolates are obtained. Currently, strain identification relies on PCR amplification, DNA sequencing and phylogenetic analysis of marker genes such as 16S rRNA or DNA gyrase B. Here, we describe the use of the intergenic spacer region 1 (ITS1) for the rapid, inexpensive and accurate typing of bacterial strains belonging to the Enterobacteriaceae. The method was validated using six cultured Enterobacteriaceae strains isolated from oak trees. The ITS1 and gyrase B genes of five sub-isolates of each strain (n=30) were amplified using PCR followed by sequencing of ITS1 and gyrB amplicons. The number and size of ITS1 amplicons for each sub-isolate was subsequently determined using both 3% agarose gel electrophoresis and polyacrylamide gel electrophoresis. Each bacterial species was found to have a unique ITS1 profile (between 3 and 7 amplicons). The validated technique was subsequently used to screen a further 270 sub-isolates derived from 54 Enterobacteriaceae strains, providing accurate species specific profiles of ITS1 amplicons. The method was found to have equivalent sensitivity to PCR amplification and sequence analysis of the DNA gyrase B sequencing gene, but with significantly reduced processing time and cost.
Evaluation of a commercial semi-selective culture medium for detection and isolation of putative necrogenic Enterobacteriaceae associated with Acute Oak Decline

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First described in 2009, Acute Oak Decline (AOD) is a disorder of native Oak (Quercus robur and Q. petraea) in the UK. Investigation into putative causes revealed many bacterial taxa, both Gram-negative and Gram-positive, but the frequent and consistent occurrence of Gibbsiella quercinecans and Brenneria goodwinii (both Enterobacteriaceae) in only symptomatic oak tissue identifies them as having a possible causal role in the condition. It is therefore of key importance that detection and identification of these bacteria is completed rapidly, especially when dealing with numerous samples. The effectiveness of Gassner agar to permit the selective growth of bacteria relevant to AOD (Enterobacteriaceae) was investigated. Fifty strains representing 21 species were used to test the medium including: Brenneria, Gibbsiella, Lonsdalea and Rahnella. There was a highly significant reduction in the growth of all the Gram-positive bacteria compared to the members of the Enterobacteriaceae (P<0.001), with only some of the Brenneria strains showing slightly reduced growth. Interestingly each species produced different colour changes in the selective medium: After 24 h G. quercinecans produced an orange colour but Rahnella spp. produced a very dark blue colour while B. goodwinii produced the dark blue only after 48 h. Although this medium is not specific for the isolation of G. quercinecans, it is useful to suppress species of no interest thereby reducing time and effort in the identification of relevant species. Further tests are required, but it may be a starting place for the production of a species specific selective medium.
Development of a real-time PCR assay for rapid identification of *Brenneria goodwinii*, associated with Acute Oak Decline of *Quercus* spp. in the United Kingdom

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Acute Oak Decline (AOD) is a complex disorder of native Oak species, *Quercus robur* and *Q. petrea* in England. Recent studies have identified two Enterobacterial species, *Gibbsiella quercinecans* and *Brenneria goodwinii* and a buprestid, *Agrilus biguttatus* associated with AOD. Culturing techniques and DNA sequencing of informative housekeeping genes are currently used to identify AOD associated bacteria. However, these techniques are labour intensive and expensive. The aim of this study was therefore to develop a rapid, specific and reliable molecular diagnostic assay for the identification of *B. goodwinii*. In order to achieve this, a large and comprehensive DNA sequence dataset based on gyraseB gene sequences was established for species of *Brenneria* and related genera within the *Enterobacteriaceae*. This DNA sequence dataset was used to identify potential species-specific DNA sequence targets for *B. goodwinii*. Using a commercial facility (PrimerDesign) *B. goodwinii* specific oligonucleotide primers and fluorescently labeled hydrolysis probes were developed. To validate their specificity for *B. goodwinii* a series of specificity tests were conducted on the LightCycler® real-time PCR platform using DNA from representative species of *Brenneria* including *B. alni*, *B. nigrifluens*, *B. rubrifaciens*, and *B. salicis*. for comparison. Additionally, the limit of detection (LOD) and quantification framework for the assay was developed by preparing standard curves based on *B. goodwinii* samples of known DNA concentrations. Results from these studies indicate that the primers and hydrolysis probe have good specificity toward *B. goodwinii* and that the assay is able to detect very low amounts of *B. goodwinii* DNA.
An episode of Acute Oak Decline (AOD) has recently been identified in Britain on native oak (Q. robur and Q. petraea) and has a rapid effect on tree health. Since 2008, numerous bacterial strains, representing several novel species, have been isolated from necrotic lesions and fluid exudates of symptomatic trees in Britain. The most frequently isolated species belong to Gibbsiella quercinecans and Brenneria goodwinii. Most of the bacterial strains isolated are Gram-negative and belong to the family Enterobacteriaceae. Species belonging to this family are difficult to identify. MLSA (multilocus sequence analysis), based on partial sequencing of 3-5 protein-encoding genes, is a robust and reliable method for identification, classification and phylogenetic studies of the Enterobacteriaceae. However, for high through put, samples are screened by sequencing the gyrB gene only, which is also commonly included in MLSA schemes. Phylogenetic analyses of gyrB sequences from symptomatic oak indicated that some of the frequently isolated strains may belong to novel species within the Enterobacteriaceae. The aim of this study was to characterise strains recently isolated from symptomatic oak in the UK and USA using a polyphasic approach based on MLSA of partial gyrB, rpoB, infB and atpD sequences, DNA relatedness studies and phenotypic assays. Phylogenetic trees generated from the concatenated MLSA data revealed that the strains form constitute seven novel species in three genera, Rahnella, Gibbsiella and Brenneria. The role of these novel species in the current episode of AOD has yet to be determined.
The Development of a Phage Therapy for the Control of the Causal Agent of Horse Chestnut Bleeding Canker, *Pseudomonas syringae pv. Aesculi*

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Bleeding canker of horse chestnut trees poses a great threat to the species in the UK and the rest of Europe. A large number of trees in the South East of England have the disease and as yet no control strategy has been found. Without appropriate intervention we may face the loss of an amenity species. To identify novel control strategies requires considerations of new therapies, especially since antibiotic usage is restricted. Phage therapy offers the opportunity for a natural, chemical-free biocontrol agent.

A number of bacteriophages from diseased and non-diseased tree material from the South East of England were isolated. These have the ability to infect and lyse all of the European isolates of the causative agent of this disease, *Pseudomonas syringae* pv. aesculi as well as some other syringae pathovars but importantly have shown they are unable to infect beneficial bacteria such as *Pseudomonas fluorescens*. A subset of 4 of these phage were further characterised and discovered to be tentatively belonging to the order Caudovirales and the families Myoviridae and Podoviridae, based on TEM and genetic sequencing results.

These phage isolates were evolved in co-evolution experiments with the host to create new genotypes to combat bacterial resistance to the therapy. These evolved phage have demonstrated a greater ability to combat bacterial resistance in, in vitro killing curve experiments; This will hopefully mean the development of a viable phage therapy strategy for the control of bacterial bleeding canker.
OptiGene Limited was formed in 2008 to develop and deliver advanced molecular diagnostics solutions for applications across a range of market sectors.

The goal was to take the power of molecular diagnostics out of the laboratory to enable testing at point-of-application. OptiGene has brought to market a number of innovative products that support sensitive and specific detection of bacteria and viruses for use in the fields of plant health, food safety, veterinary medicine, environmental monitoring and healthcare. These products support rapid molecular detection from crude samples by employing isothermal amplification of DNA and RNA.

OptiGene has exploited its expertise in both instrument design and enzymology to develop a sophisticated open platform that will support all isothermal amplification methods. State-of-the-art instrumentation is supported by specially-designed plastic strips that have lockable caps and reagents that offer world-leading reaction rates. Until now, ultra-sensitive molecular detection that has been constrained to laboratory use by highly-qualified personnel and taking hours to complete can now be deployed to point of application and run with very little training, producing results in single minutes.