CFP Internship Diary

I applied to the CFP internship because I was keen to start a career in tree pathology research. The internship would be a perfect opportunity to gain experience working in the lab and in a state-of-the art quarantine facility. It also sounded appealing as I would be able to contribute to a major research project and be fully immersed in a research environment.

The Holt Laboratory at Alice Holt

In my internship, I supported a project studying the effects of *Xylella fastidiosa* on UK tree species (hazel, cherry and oak). One of the aims of the project is to access the ability for different strains of the bacteria to establish and systemically colonise the plant. As the pathogen is not found in the UK, experiments can only be conducted in the quarantine laboratory (Holt Lab). Therefore, I spent the majority of my time in the lab and gained hands-on experience with lab techniques involving microbiology, pathology and molecular biology.

October

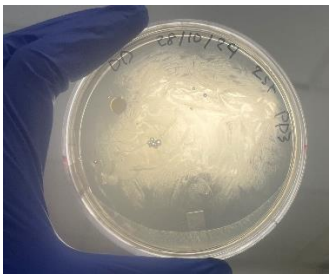
In the first two weeks, I spent most of the time completing mandatory trainings and lab inductions. I also read papers and standard operating procedures (SOPs) to familiarise myself with the topic and protocols. After that, I finally had hands-on experience in the lab. To start, I recorded phenotypes of the oak saplings in the lab and learnt taking leaf samples for analysis. These oaks were inoculated with *X. fastidiosa* and were kept in the growth chamber for long-term monitoring. This was the second time-point, in which we were going to take some samples to test for the pathogen. I then learnt extracting DNA from the leaf samples using the DNeasy Plant Kit and running quantitative PCR (qPCR) to detect the bacteria.

Oak saplings inoculated with *Xylella fastiodsa*

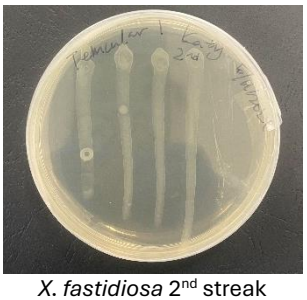
November

In the start of November, I learnt making specialised media for *X. fastidiosa* and poured agar plates. I then had the chance to culture four strains of *X. fastidiosa* (Temecula 1, De Donno, IVIA 5235 and CFBP 8173) on different media using a special technique. In contrast to the streak plate method, the culture should be either spread continuously with circular or horizontal movements.

After a month of incubation, I checked on the plates again and the bacteria grew! I then resuspended and plated the culture to do the second streak, which took two weeks to grow. I resuspended the bacteria to inoculate *Nicotiana benthamiana* using the pin prick method and performed a serial dilution to later on count colonies. This is a separate project, alongside my supervisor's PhD, as an opportunity to apply the techniques I had learnt and manage a project independently.



X. fastidiosa 1st streak – the 'creamy' morphology indicates biofilm being produced



X. fastidiosa 2nd streak

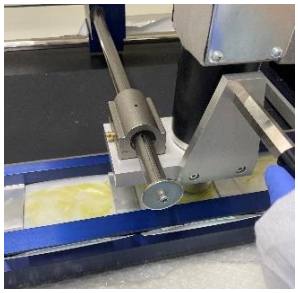
Tobacco (*Nicotiana benthamiana*) plants inoculated with different strains of *X. fastidiosa*

As a side project to optimise the protocol for *X. fasitidiosa* detection, we compared the use of water and CTAB as buffers to homogenise samples and extract DNA. To investigate this, we collected oak leaves from the field and inoculated them with the bacteria of different concentrations. The leaves were then left to incubate for 48 hours at 20℃ and frozen for subsequent processing.

Inoculated oak leaves

Next, I learnt techniques to shave cherry and hazel stems that had previously been cut to analyse. The bark of the stems was removed and the remaining tissues were shaved. The shavings were then put into extraction bags and homogenised by the homogeniser. After that, I carried out DNA extraction using the Kingfisher robot and measured the DNA quality using the Nanodrop. I then performed qPCR to detect the bacteria.

Shaved cherry in extraction bags



Samples being macerated by the homogeniser

The Kingfisher robot for DNA extraction

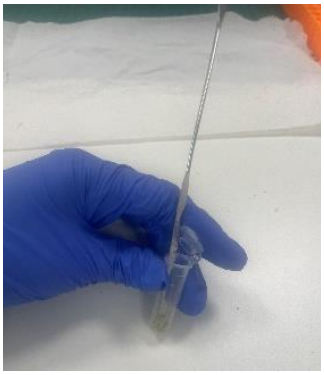
RHS PhD Symposium at Wisely

To wrap-up the month, my supervisor and I attended the Royal Horticultural Society PhD Symposium in Wisley, in which she gave a presentation on her PhD. It was a great opportunity to understand her project from a different perspective and learn more about other PhD projects. I also had the chance to mingle with the speakers.

December

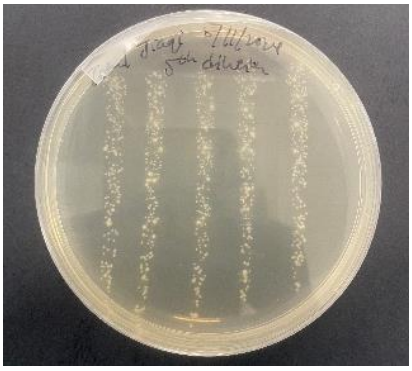
December started with a new set of experiment analysing metabolomics between healthy and infected trees using mass spectrometry. I learnt how to crush the cherry and hazel samples into fine dust and extract metabolites using methanol. They were then sent to the University of Birmingham for High-Performance Liquid Chromatography – Mass spectrometry (HPLC-MS) to annotate and analyse secondary metabolites. I also had the chance to self-learn new software MZmine and SIRIUS to analyse a few samples.

It was also time to check on the serial dilution plates I did earlier on in November. They worked perfectly and the bacteria grew well! I then picked plates with countable number of colonies and performed colony-forming unit (CFU) counts which would give an idea of the number of bacterial cells being put into the plant.



Crushing samples with a spatula

The sonicator that releases ultrasound to facilitate metabolite extraction in the samples

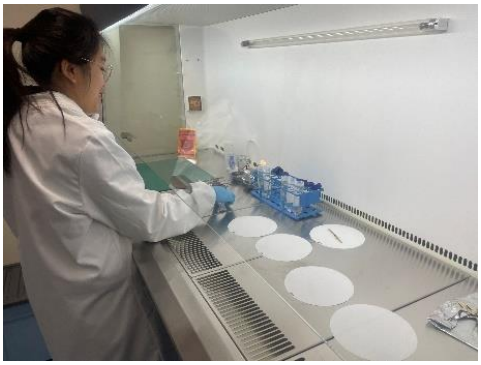


X. fastidiosa colonies in the 5th dilution

January

Coming back from the Christmas break, we began taking down the oaks that had been monitored throughout these months. After recording the phenotypes, the main stems were cut and put into extraction bags for further processing. Some were shaved and homogenised for DNA extraction and subsequent qPCR, while others were used for isolating the bacteria from the plant. Isolation from plant materials is one of the most

challenging experiments throughout my internship. Procedures had to be done under the laminar flow and everything in contact with the sample had to be sterilised. Despite being a long and stressful experiment, it trained my ability to work under pressure and time constraints. I also assisted in giving training to Forest Research staff by my supervisor on the workflow for testing *X. fastidiosa* samples.



The first step is surface sterilisation – sticks were washed with soap and put into bleach, ethanol and water, each strictly for 2 minutes

Surface-sterilised sticks were shaved and put into extraction bags for maceration

Plating macerates onto agar plates to let the bacteria grow

Buffer added into the extraction bags ready for homogenisation

Lastly, I processed the oak leaf samples for the water vs CTAB experiment. The samples were processed and DNA was extracted using the Kingfisher robot. We encountered problems with qPCR, as we unexpectedly did not observe any positives in the samples extracted with water, and had to troubleshoot. After a few trial runs, we found out the problem was that one of the reagents did not work with the water protocol. I enjoyed the process of finding out the cause of the problem and discussing solutions. It was a good chance to train my problem-solving skills in research.

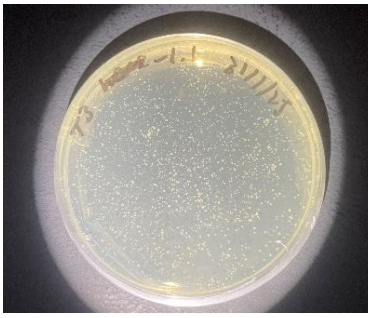
Once done troubleshooting, we continued with the experiment. We found out that samples extracted with water had a higher DNA concentration, which potentially explained their lower Cq values in qPCR. Thus, we concluded that although water is less effective in removing inhibitors, the water protocol works better than CTAB in giving earlier qPCR results. Since some cherry and hazel samples were previously extracted with CTAB, we had to re-extract DNA from the homogenised macerates with water. I learnt how to wash away CTAB in the macerates with water and tweak the centrifuge settings to ensure good quality of extracted DNA.

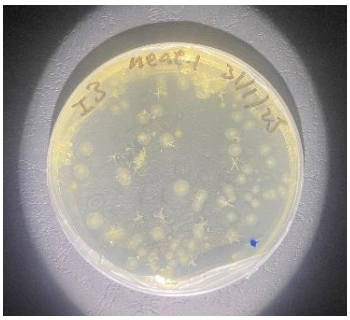
February

Tobacco stem to be processed

It was time to process the tobacco plants that were previously inoculated. Same as the oaks, I took down the tobacco and tested them using qPCR, which all (except the controls) came positive! Isolating the bacteria from tobacco felt easier as I had previous experience with the oaks and did not need to shave them. However, it was still challenging as I had to handle many plates by myself under time constraints. Nonetheless, the experiment went well and I successfully isolated *X. fastidiosa*! We then performed colony PCR and gel electrophoresis to verify if the colonies observed were the bacteria. Identifying *Xylella* morphologies and hence picking up the correct colony were tricky (but fun!) as they sometimes varied.


Over 150 plates for isolation


X. fastidiosa successfully isolated from tobacco – typical small, whitish, circular morphology


The 'fringe' morphology of *X. fastidiosa*


Tobacco plants

The tobacco experiment is certainly one of the highlights of my internship, as I was able to apply all the techniques having learnt and manage the project independently. Although the plants did not look very well, I gained experience with monitoring plant growth in the growing chamber, such as watering regimes and pest control. I also had the chance to plan own experiments and manage my time. I learnt that preparing ahead, such as having everything ready and labelled beforehand, and keeping track of time are important. This can smoothen the process especially when the experiment is time-consuming and cannot be stopped half-way.

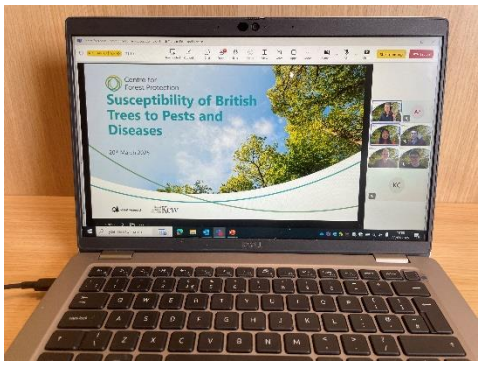
March


A lovely garden tour at Wakehurst

At the beginning of March, we had an intern day in Wakehurst. It was a great opportunity to meet other interns and CFP staff and visit the site. We attended insightful talks from scientists on their research in seed conservation, followed by a tour in the Millenium Seed Bank. It was eye-opening to visit the world's largest plant species genetic resource and learn about the

process of conserving seeds. We then had a fascinating garden tour, in which I understood more about the management and conservation practices to house such diverse plant species. The day ended with a reflection session on the internship.

As the lab was closing down and my supervisor's work was coming to an end, we finished our lab work and moved on to computer work. I spent the remaining of March putting the phenotype photos together, organising the datasets using Excel and analysing data with R.



Getting ready for the presentation!

To draw an end to my internship, I presented my work in the CFP webinar about the water vs CTAB experiment. The audience was a mix of policy-makers, practitioners, scientists and students, thus I simplified the content and explained the lab techniques involved. It was a good opportunity to enhance my public-speaking skills, share my internship experience and learn about other research projects.

Reflection and What Next

Overall, I enjoyed my internship and it has been a rewarding experience. I gained experience with different lab techniques and valuable knowledge working with *X. fastidiosa*. I also enhanced my lab skills, including time management, attention to detail and healthy and safety, which are crucial in a lab role. Working closely with a PhD researcher, I had a taste of the day-to-day life of a PhD and what it is like doing research. Collaborating as a team in the lab, I honed my communication and collaboration skills to carry out the experiments more efficiently. Moreover, working at Forest Research, I was able to meet like-minded people and establish connections. This internship reinforced my interest and confidence in pursuing a research career. Thus I will be looking for research assistants and potentially PhD positions in related fields.