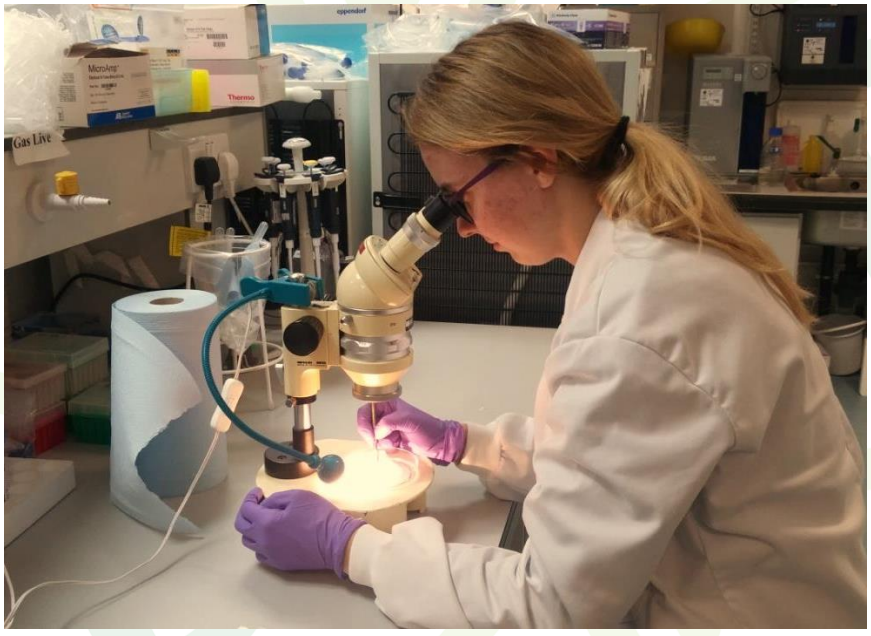
The detection of '*Candidatus* Liberibacter solanacearum' in psyllids and aphids

I have spent my summer reviewing detection methods of psyllids and aphids for '*Candidatus* Liberibacter solanacearum' (CaLsol) at Science and Advice for Scottish Agriculture (SASA), in Edinburgh. My project ran alongside ongoing research at SASA developing diagnostic molecular tools for psyllid identification. There are 3 psyllids species that are confirmed vectors of the phloem-limited bacterium CaLsol. CaLsol occurs as 5 geographically distributed haplotypes. Haplotypes A and B are found in the US and New Zealand and infect potato plants. They are on the EPPO A1 list of quarantine pests and are not present in Europe. Symptoms can include necrosis of internal tuber tissue in potatoes that, upon frying, gives rise to a characteristic striated morphology that earned the disease name: 'Zebra Chip'. Infected crops are rendered unmarketable and diseased plants rarely survive, posing a real economic problem. Haplotypes C, D and E mainly infect carrots and celery in Europe. Haplotype C is present in Scandinavia and is transmitted by the psyllid *Trioza apicalis,* which co-occurs with several aphid species for example, the carrot aphid *Cavariella aegopodii*. *T. apicalis* also causes physical detriment to plants by feeding and thereby rupturing the phloem tube. Psyllids and aphids are from the same suborder, sternorrhyncha, both feeding on phloem and classed as hemiptera (meaning true bugs). They have piercing mouth parts which can feed on phloem and can potentially uptake phloem limited bacteria such as CaLsol. In this project we wanted to test whether, as phloem feeders, aphids could also be carriers of CaLsol and therefore could be used as an alternative system for monitoring CaLsol infection in areas where psyllids are scarce.

We obtained psyllids and aphids from suction traps in Sweden and the UK and tested for the presence of CaLsol using real-time PCR. CaLsol positive samples were then sequenced at three distinct regions of the genome: 16S, 16-23S and 50S rRNA genes. Sequences were aligned with reference sequences from each haplotype. Single nucleotide polymorphisms between each of these regions could be compared to determine a haplotype. These regions were also sequenced from a vector psyllid (*Bactericera cockerelli*) that was reared on CaLsol positive plants to test the haplotyping procedure. Some aphid species were weakly positive for very low amounts of CaLsol, but the quantities were too low for successful haplotype sequencing.

To further explore infection within the CaLsol positive *B. cockerelli* psyllids, I designed a new method for DNA extractions from the head, thorax and abdomen to determine whether CaLsol could be detected from each segment. It was thought that CaLsol would be primarily found in the abdomen where the gut is located as psyllids initially uptake the bacterium by feeding on the phloem. The bacterium is then passed on to the salivary glands for transmission. For the infected vectors, CaLsol was detected in all segments at high concentrations suggesting that CaLsol is spread throughout the body of the psyllid and not localised only to the gut.



Performing DNA extractions on psyllids and aphids

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The detection and haplotyping of CaLsol has worked successfully in psyllid vectors. However, aphids have yet to be confirmed as carriers of CaLsol. Aphid specimens produced weak positives in comparison to known vector psyllid species. This suggests that aphids could be carriers of CaLsol but not vectors, and could be used as indicators to monitor CaLsol spread.

This project has allowed me to practice a wide range of laboratory skills and further expand my knowledge of pathology. I enjoyed working in a laboratory environment, learning new techniques and discussing new areas of research. I feel my confidence has improved alongside my enthusiasm for plant pathology. I would like to thank BSPP for funding and to thank SASA for the opportunity to work at the fore-front of crop protection. I now feel better equipped to undertake my heavily project driven final year at university with a renewed interest in plant pathology.

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