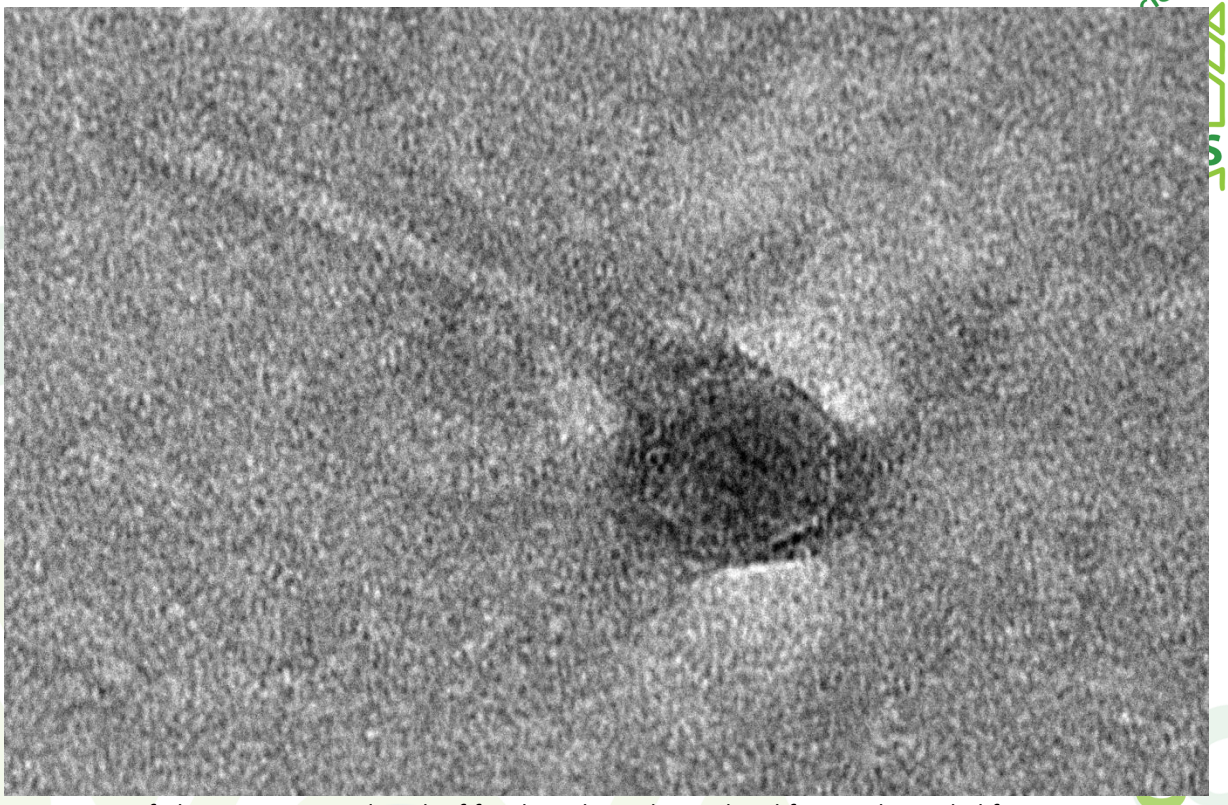
Collection and characterisation of native bacteriophage; a potential novel biocontrol agent to treat bacterial canker of *Prunus*

Cherry (*Prunus avium*) production in the UK is a rapidly growing sector, yet current yields are limited by pest and disease. One of the main diseases of cherry is bacterial canker caused by *Pseudomonas syringae* pathovars *syringae* (*Pss*) and *morsprunorum* (*Psm*). New regulations mean that using copper, the most effective current treatment, as an antimicrobial will no longer be permitted. An alternative control strategy is required by growers to manage the disease. Phage therapy, using bacteria-killing viruses to prevent or cure an infection, offers great potential as a targeted, non-toxic biocontrol agent. This summer under the supervision of Professor Rob Jackson at the University of Reading I carried out a project to collect, isolate and characterise bacteriophage as a potential biocontrol treatment against *P. syringae* infections of *Prunus* species.

In July I travelled to East Malling Research (NIAB EMR) in Kent to meet with Dr Robert Saville, Research Leader in Pest and Pathogen Ecology. We visited the on-site genebanks and demonstration plots, a commercial farm, and the National Fruit Collection (NFC) at Brogdale in order to sample soil and leaves from a variety of *Prunus* species and cultivars, including cherry, plum, apricot and damson. As bacteriophage are one of the most abundant entities on the planet, phage specific to the target host can be readily isolated wherever the host bacteria can be found, for instance in leaves and soil around host plants. Trees sampled were also assessed for the symptoms of canker, which manifest as large, dark, gummy wounds on the trunk and branches, and shot-holed leaves. Definitive diagnosis could not be made however, as canker wounds dry in summer becoming less visible, and shot-hole leaves can also be indicative of a fungal infection. I was also supplied with three host strains (*Pss*, *Psm* Race 1 and *Psm* Race 2) by PhD student Michelle Hulin, whose research investigates the host specificity of *P. syringae* pathovars on *Prunus* species.

In the Jackson lab at the University of Reading the samples were prepared for phage isolation by adding soil or leaf to tubes containing saline buffer which were then vortexed, centrifuged and filtered to remove any bacterial cells. This filtrate was then plated with each of the three *P. syringae* strains using a double-agar plaque assay, later modified to increase the volume of filtrate in the top layer of agar to increase the sensitivity for very low phage concentrations in the samples. The presence of phage in the sample results in circular clearings in the agar called plaques. Using this method, I was able to titre, isolate and purify 20 (18 from soil and 2 from leaf) different plaque morphologies in total. The higher proportion of successful isolations from the soil samples is likely due to the harsh environment of the phyllosphere destroying phage through solar irradiation and desiccation. In addition, I found no significant difference in the presence of phage from symptomatic trees versus asymptomatic trees. The results from the host range experiments show that some phage were strain specific whilst others were able to infect all three. All phage were stored for future use.

Several plaque morphologies were selected for further characterisation. Amplification was achieved by calculating the minimum multiplicity of infection and enriching phage with a high volume and concentration of host cells in a broth, incubating, then filtering out the bacterial cells. These stocks were used in transmission electron microscopy to morphologically characterise the phage (see figure), my personal highlight of the studentship. The phage DNA was extracted and stored for further characterisation. The growth curve for one phage was also examined.



TEM image of phage, ~240nm in length of family *Siphoviridae*, isolated from soil sampled from the base of a Stephen's seedling Cherry tree at Brogdale National Fruit Collection. 88000x magnification.

I feel that this ten-week project has given me invaluable insight into the UK fruit industry and the role that plant pathologists play in ensuring future food security. I have developed my understanding of how a research project is conducted, from the day-to-day activities of preparing overnight broths and pouring plates, to field sampling, phage isolation techniques and electron microscopy. I aim to continue this research to sequence the phage, trial the application of phage to trees, investigate delivery techniques, phage cocktails and persistence in the environment to determine their efficacy as a biocontrol agent.

It's been fascinating to work on the emerging biotechnology of phage therapy which shows so much promise for managing plant (and human) disease. I would like to thank the BSPP, Dr Robert Saville and Michelle Hulin from NIAB EMR, GH Dean & Co., Brogdale NFC and everyone from the Jackson and Ian Jones labs for making this project possible.

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