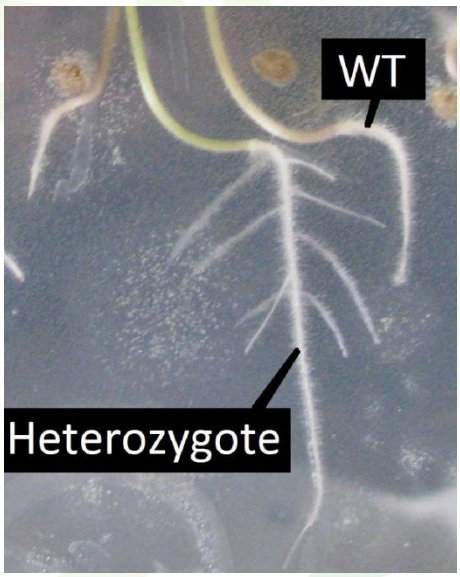
How Cucumber Mosaic Virus infection of tomato plants affects bumblebee pollination behaviour

During my summer research project my aim was to investigate the phenomenon that infection of a host tomato plant by cucumber mosaic virus (CMV) appears to increase the frequency and duration of pollination by bumblebees. I was very fortunate to be working under the supervision of the Carr virology lab at the Department of Plant Sciences in Cambridge; the lab had recently published a paper illustrating their discovery of the unusual bee-plant-virus interaction, titled 'Virus infection of plants alters pollinator preference: a payback for susceptible hosts?' Bees are not vectors for CMV, so it was therefore hypothesised that virus susceptibility could be maintained in a population through enhanced pollinator attraction. CMV infected plants were shown to produce fewer seeds than mock-inoculated plants when in the absence of bee pollination, so one would predict that eventually resistant plants would come to dominate and become fixed within a population. This however could be counteracted by the fact that when virus infected plants are pollinated by bees seed production is recovered.

Seeds in tomato fruits result from self-pollination and bumblebees help to maximise seed number by 'buzzing' the anther cone in the centre of the tomato flower. The bumblebees are rewarded with pollen (a protein source for the colony) while they sonicate the anthers and enhance pollen delivery to the stigma. However, during buzz-pollination, it is possible for the bee to facilitate cross-pollination as it can bring pollen from another flower. Pollination experiments were carried out to see if CMV-infection affected the rate of cross-pollination. Experiments designed to track the movement of pollen by bumblebees from virus-infected tomato plants (expressing the green fluorescent protein and neomycin phosphotransferase marker genes) to non-infected wildtype plants (and vice-versa) were carried out. My task was to devise a rapid screen to identify seed resulting from cross-pollination within a population of seeds resulting mainly from self-pollination. I developed a high throughput screening for resistance to kanamycin on large agar plates grown vertically to detect kanamycin resistant heterozygotes produced by outcrossing. The vertical growth method allows for very easy detection of resistant heterozygotes by observing differences in root length and looking for lateral root growth (pictured below).



PCR analysis using GFP, NptII (kan resistant) and EF-α (control) primers confirmed that kanamycin resistant heterozygote seedlings showed the same DNA bands as the pollen donating transgenic parent plant. GFP expression in the crossed seedlings was confirmed by epi-fluorescence microscopy. This demonstrated that marker genes could be transferred between plants by bees via pollen in glasshouse experiments. Screens of these seeds also showed a consistently high rate of germination, an average of 93% germination rate of seeds was observed.

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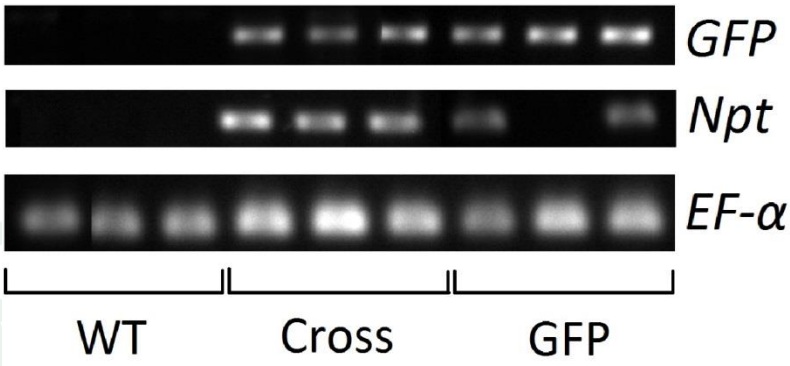
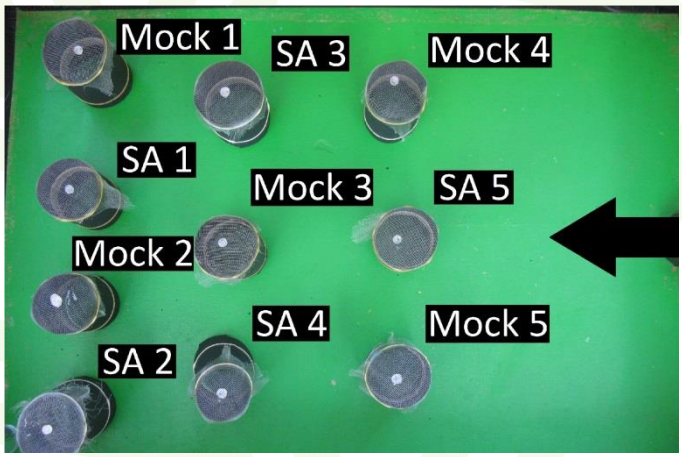


Another aspect of my project was to investigate the possible role of salicylic acid (SA), a key regulator of plant defence, on bee behaviour as it is produced in high quantities in response to CMV infection. I carried out preliminary experiments to see if plants treated with SA were more attractive to bumblebees. SA-treated and mock-treated plants were placed under opaque towers within a bee flight arena. Each tower had a small cup containing sucrose solution on top. Single bees were released into the arena and allowed to choose a cup from which to feed. I found that bees preferred to feed from towers containing the SA-treated plants indicating that volatiles from SA-treated tomato plants are attractive to bumblebees. The results of this preliminary test are very promising and will be followed up with larger scale experiments.



I am very grateful, both to the fantastic scientists who supervised me and assisted me greatly during my time in their lab and to the BSPP who's funding gave me the opportunity to experience real lab work, including lab techniques and experimental design. The results attained from the experiment are very promising and I wish the Carr lab the best of luck with continuing their investigation in my absence.

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