How to win an evolutionary battle: Exploring plant defence response against aphid attack

Biochemical Society Summer Vacation Studentship

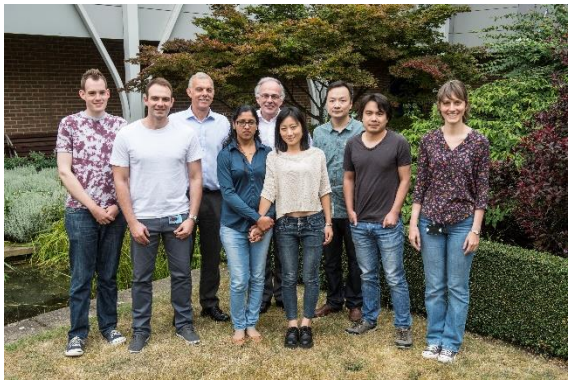
By James Canham

Arrival at the world renown John Innes Centre (JIC)

I was greeted by my project supervisor, Tony Miller and day to day manager, Tom (Ph.D. student), familiar faces as I had been fortunate enough to spend a little time working with them, on the rare occasion my coursework allowed breathing space, throughout the spring semester. Tom is interested in plant-aphid interactions and would like to deduce the underlying mechanisms which determine whether a plant is a suitable host to aphids or is capable of mounting a successful defence against the common crop pest.

Tom's work is a collaboration effort between the labs of Prof. Dale Sanders (JIC director) and Prof. Saskia Hogenhout. Their combined research couples 2nd messenger signalling molecules and plant-aphid interactions. I will be working primarily in the Sanders lab which is managed by Dr. Tony Miller, my project supervisor. A short conversation with Tony revealed he is from the same small Norfolk town as I am; quite a coincidence given the diverse nature of those working at JIC. Within the Sanders/Miller lab, 3 post-doctorate researchers, Paloma, Kaliani and Yi as well as Yanshu (visiting Ph.D. student) have proven to be very accommodating, sharing vital information such as where the microwaves are to heat lunch and which cycle racks give the easiest access to the office! I know that their other talents, namely their scientific research skills, will be of great help in the coming weeks.

I will have weekly meetings with Tony and the group to update them on my work. I also have weekly meetings with Prof. Sanders on a Friday morning which will certainly keep me on my toes. Meetings with Prof. Hougenhout depend on queries that arise during the coming weeks. I will be required to give a talk to their group at the end of my summer placement.



The Sanders/Miller lab members. From left: Tom (day to day manager), James (me), Tony (lab manager and supervisor), Kaliani, Dale (JIC director), Yanshu, Yi, Chengwu, Paloma.

Reading, site tour, more reading

Very kindly, Tom described his project to me in ~~all~~ most of its detail. It allowed him to practice a presentation of his work that he would give in the USA whilst visiting external collaborators next week. It gave me a much needed insight into the incredible attention to detail and breadth of his work. There were a number of occasions Tom stated, 'I'll send you that paper' throughout. Before I had the time to logon to my computer, half a dozen papers were attached to an email. I knew it would be a busy first week.

The Norwich Bioscience Institutes cover a considerable area with many features such as glasshouses, office and seminar buildings and, of course, research laboratories packed onto the site. The site tour threw up some very important dos and don'ts and I knew my attention would be tested. For example, genetically transformed plants are grown in a restricted entry building. After choosing the correct lab and day length room, the room specific lab coat and hair net must be worn. 'You must come here before you visit the insectary', Tom said. I will be carrying out work with plants and aphids. I will need to visit both places prior to experimentation. If I happen to visit the insectary before the collecting the plants, I cannot enter! The insectary itself has three levels of security, each with its own specific set of regulations.

Fish pond and gardens at JIC.

Confirming genotypes with phenotypes

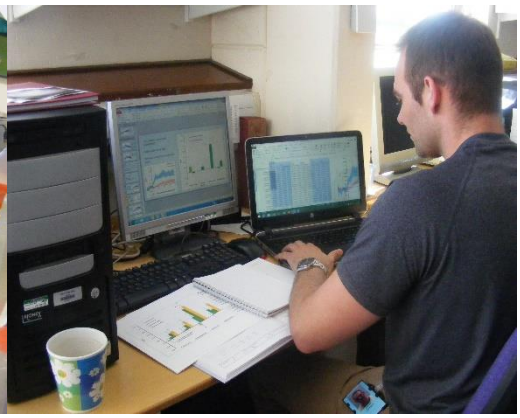
My first experiment will cover a small part of a bigger picture but will give me much needed experience handling and propagating *Arabidopsis* seeds. I will screen for a low germination phenotype in salt stress conditions of a protein kinase knockout, *cipk3-1*. Comparing the T-DNA insertion knockout genotype to a wild-type genotype, should help to confirm the CIPK3-1 knockout we will be working with for some future experiments.

To draw meaningful conclusions, multiple repeats were necessary. Next week, these seeds will need to be viewed under a stereo microscope and counted individually.

When the supervisor is away, the mice will…Work!

Whilst Tom is visiting collaborators, I will be under the guidance of Paloma, a post-doctorate researcher who is concerned with zinc mobilization and localization in wheat grain. We have been tasked with extracting total RNA from *Arabidopsis* leaves exposed to aphids and compare them to a control. This will give us an indication of the transcriptome changes aphids induce in the plant. As we know *Arabidopsis* is a viable host for these particular aphids, it is very interesting to see if our protein kinase, CIPK3-1, is being differentially expressed.

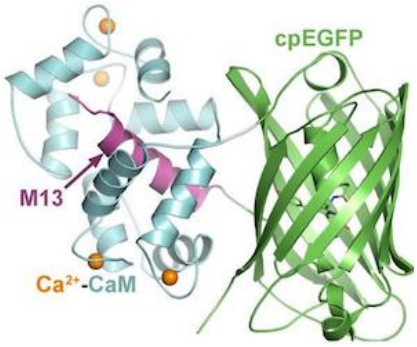
After 4 mornings on the stereo microscope I could collate all the data from the cipk3-1 germination assay. All in all, that is over 12,500 seeds sterilized, plated and counted. I then tabulated this data onto excel and exported it into a stats package were I was able to draw some meaningful conclusions. And, happily, they agreed with a previously published paper where similar experiments were conducted…Very pleasing!



Working at my lab bench and on my computer(s). My time will be roughly equally divided between the two locations as I collect and interpret data.

Making plants talk

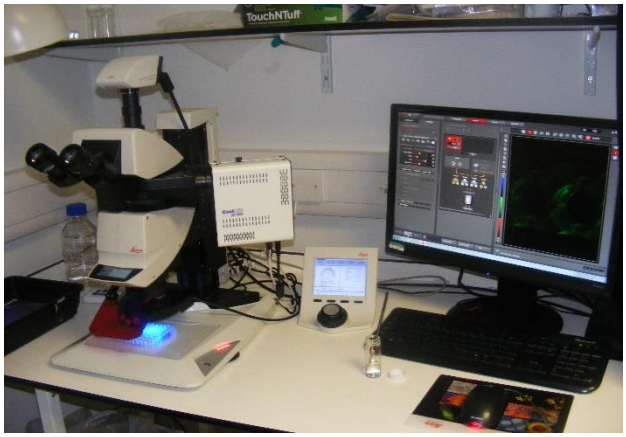
I will be using a genetically encoded indicator to study transient Ca^{2+} concentrations. These GFP reporters were produced by Tom's American collaborators and offer an excellent opportunity to visualize the plant defence mechanism in real time and in a non-intrusive manner.



GCaMP reporter molecule.

Establish parameters, protocol and populations

I have been busy recording plant-aphid interactions real-time on a stereo microscope using reporter plants. We initially used wild-type plants and compared treatment and no treatment control. After some trial and error, Tom put together a workable screen which would allow for accurate recording and multiple repeats. Data analysis was optimized and we began to standardize every aspect of the experiment.



Leica M205 microscope at JIC.

This standardization included producing aged aphid and plant populations. *Myzus persicae*, the aphid species we are using, generally reproduce asexually to produce female offspring under ideal conditions. Female adults give birth to, on average, 1 nymph every 24 hours. Producing aged populations therefore requires placing adults on a plant, incubating overnight, and removing the adults without disturbing the nymphs the next day. Friday would be my day for plating the *Arabidopsis* reporter plants to ensure a ready supply come imaging.

In the coming weeks I will use the wild-type as the control and test for differences in plant responses in mutant lines. This is particularly interesting as it may help elucidate the important channels involved in a compatible plant-aphid interaction.

Part 2:

A field trip to the field!

Members of the Prof. Saskia Hougenhout's lab with some additions including Tom, (day to day manager) 5th from left and myself, 4th from right.

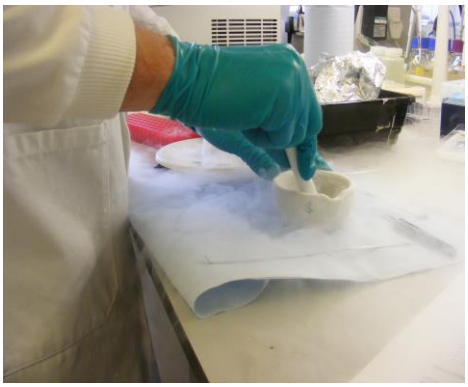
A number of JIC labs are involved in field trials of various crops with specific interests across many aspects of plant science. This week, we visited a wheat field under the operation of Saskia's group. We were on the hunt for aphids as this would give the researchers some indication of resistance in different genotypes. It gave me an opportunity to meet the other group involved in Tom's work and see an applied example of where the science may lead. A very enjoyable, sun filled afternoon.

DNA is simple. RNA is more difficult.

I have been very busy in the lab juggling my time between imaging and molecular work. Despite some initial issues, namely with the chemical contamination, I seem to have got on top of running PCR amplification protocols.

PCR machine in the Miller lab. A piece of technology that is easily taken for granted by those unfamiliar with previous, more laborious methods.

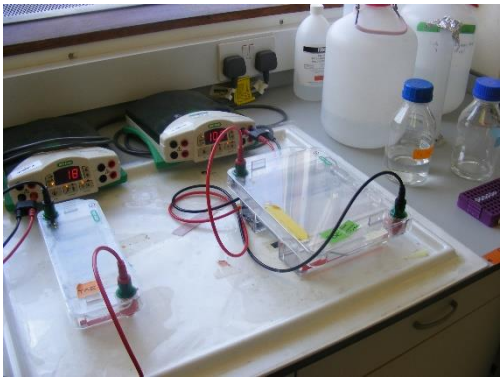
I particularly enjoy investigating transcriptomic changes. This involves flash freezing the plant material in liquid nitrogen and extracting and purifying RNA. Given the transient nature of RNA, it is not too surprising that it is very susceptible to degradation. Working quickly and always on ice is a necessity.



The use of liquid nitrogen is performed under supervision in the lab. It is very important to gain a snap shot of the transcriptional changes in living material.

Working independently in the lab

A surprising revelation which has recently come to the fore during a lab meeting is the independence each member of the lab has. Although Saskia's group choose to keep a record of preferred protocols for any repeated work, each member has their own, tried and tested methods. Even differences in gel electrophoresis buffer, the voltage and preferred running time will vary between every member of the group! Although it is quite a quirk, it can make it a little difficult to be decisive and commit to a particular protocol.



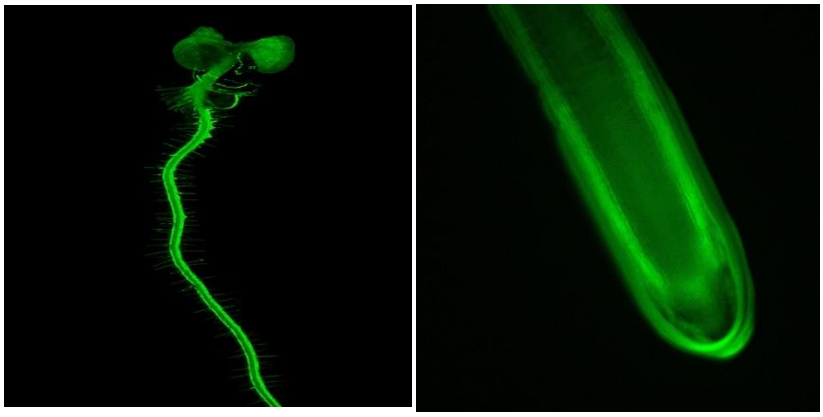
Gel electrophoresis machinery. Used in the lab primarily to separate DNA and/or RNA fragments by size.

Lesson learnt. Record the protocol accurately. Revisit the protocol to state the level of success. Add alternative methods.

My responsibilities are evolving

This week the lab has welcomed a new member. Evvia is a talented college student with particular interests in biochemistry. She won a summer research placement and has been placed in our lab. Her work will overlap mine and so I have been tasked to bring her up to speed. It is a wonderful opportunity to communicate the science and ensure I'm very confident with all that I've learnt. I will also help design experimental assays that will allow her to collect data over the coming weeks.

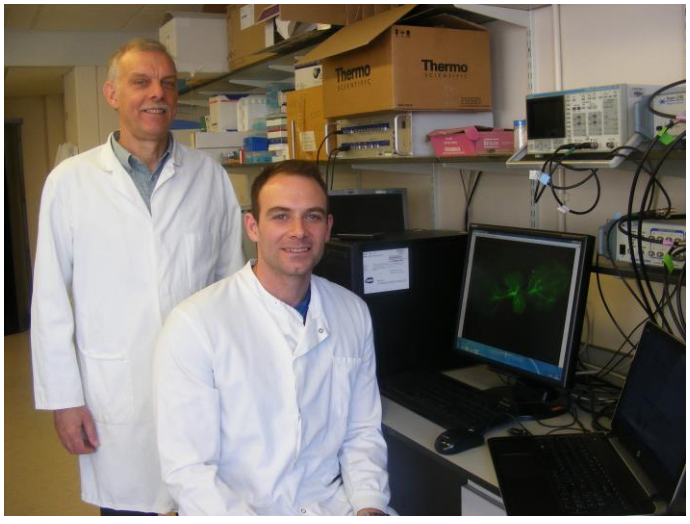
To familiarize herself to the imaging, she has been collecting some photos of the reporter plants. Some of these give us a wonderful insight into the ways plants use calcium.



GCaMP Ca^{2+} reporter plants imaged under blue light. Left is whole seedling. Right is a root tip. Fluorescence is indicative of cytosolic calcium.

My final week

I have confirmed with Tony that I will undertake my final year dissertation in the lab. Many of the skills I've learnt over the summer will become very useful once again. I am extremely grateful to Tony, Tom and all my colleagues in the Sanders/Miller lab. Their patience and encouragement has been amazing. Saskia and members of her team have been equally supportive and very forthcoming with advice. Finally, **a big thank you to The Biochemistry Society**! This summer's experience was made possible due to their wonderful support of undergraduates with the Summer Studentships.



Dr. Tony Miller and Myself (right).

Yours faithfully,
James Canham