Scholar Report

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Title of Scholarship Project:	Bees, Pesticides & Insect declines: defining the sublethal effects of pesticides on honeybees

Importance of the Research

Pollination is essential for crop production and environmental stability. In terms of the economy and politics, honeybees are the most vital contributors to the pollination of commercially grown crops (Hung et al., 2018). The value of their pollination services means that the conservation of honeybee colonies across the world is becoming increasingly important, as the need for food security grows (Sillman et al., 2021). Although the general public is becoming more aware of ways to support honeybee populations- such as by diversifying their garden flora- it could be argued that governments and farmers have greater responsibility over honeybee health.

Currently, the UK Government permits the use sulfoxaflor-based pesticides in greenhouse conditions and have stated that it has little effect on bees (GOV.UK, n.d.). However, recent research is revealing that sulfoximine products, like sulfoxaflor, begin to have sub-lethal effects on bees when used chronically (Capela et al., 2022). This possible misinformation is likely to have followed limitations in approval testing, as lack of funding and time meant that only toxic effects, occurring as soon as contact was made with the pesticide, were investigated. Whilst expanding populations mean it is vital that agricultural land is protected from pests, it is also important that pollination levels are maintained so that an unwanted negative feedback loop is not created. This research project aimed to investigate the sub-lethal effects of sulfoxaflor.

The project also had further purpose, as I worked to help some of my peers in the lab. This was done through conducting behavioural assays to assist one of the PhD student's research projects. I also analysed the data collected from these experiments (since he was not trained in the area). This helped him to have a greater understanding of the chemicals that his honeybees were being treated with, and the effects that they had. Having the opportunity to do this helped me in understanding the meaning that my work had, as I collected information for others to extend their research.

Research Conducted

Globally, in recent years, the decline of honeybee populations has become apparent (Shanahan, 2022). These losses can be blamed on biological factors such as disease or natural climate change (Shanahan, 2022). However, it could be argued that anthropogenic factors such as urbanisation, intensified agriculture and pesticide use are having the most

negative effects (Shanahan, 2022). Partial research has been done on sulfoxamine-based pesticides, however there are gaps in the knowledge of the effects on honeybees following long-term, sub-lethal exposure.

Upon starting this project, I proposed a hypothesis to be tested: Sulfoxaflor causes disruption to honeybee colonies by affecting response to both brood pheromone and queen mandibular pheromone. With this in mind, I was provided with the support to develop the skills required and obtain results, to begin answering questions surrounding this hypothesis. Various methods were used to collect the data required.

Behavioural Work:

Proboscis Extension Response Assay

The Proboscis Extension Response assay is a technique used to estimate individual honeybee sucrose response thresholds (Matsumoto et al., 2012). This is done by observing whether a honeybee begins to extend her proboscis in response to lower or higher concentrations of sucrose solution. This is useful information to collect as sucrose response thresholds can indicate whether a bee is likely to forage for pollen or nectar, which in turn provides evidence to estimate the development of bees.

Method:

A sample group of bees was removed from each cage. Prior to harnessing the bees, they were chilled for 5-10 minutes. Chilled bees were then placed into metal tubes and secured using masking tape- ensuring only the bee's head, proboscis and antennae were exposed. Once all bees were harnessed, they were left to recover for 15 minutes and then provided with water until no longer extending their proboscis, ensuring that they were full. Bees were then left at room temperature for a further 30 minutes (during the beginning of my project this time was 1 hour, however I concluded that this period was too long). After this time, testing began. Each bee was tested with 6 sucrose solutions of increasing concentrations, with a water solution being provided between.

With each solution, it was recorded if a bee extended her proboscis in response to her antennae being touched with the solution. An overall proboscis extension score was calculated at the end of the experiment. Since there were 6 sucrose solutions, scores ranged from 0 to 6. A score of 1 indicated a high proboscis extension reflex response threshold, whilst a score of 6 indicated the opposite (scores of 0 were not significant to this experiment). This data was copied from my lab book into Excel, where it was then analysed.

Retinue Response Assay

The retinue response assay relies on behavioural observations to provide assumptions about bee's QMP response (Vergoz et al., 2009). In this instance, I was testing whether bees treated with Sulfoxaflor responded differently to QMP in comparison to a control group of bees treated with water.

Method:

Bees were placed in an individual petri dish marked with 4 rings to indicate different sections. A microscope slide was also in the dish, with 10 microlitres of either ethanol or

0.01qe QMP in the centre. 4 bees were tested at a time, for 5 minutes, and recorded using a web cam suspended from above. Each bee was tested twice, once with ethanol and once with QMP. The entire experiment was done under red light, which bees cannot see in. This removed any chance of light interfering with the behavioural results, as bees are positively phototactic.

Using the footage recorded using the webcam, an online software was used to provide snapshots from every 10 seconds of the video. These pictures were then observed, and the position of bee throughout the experiment was scored. Scores ranged from 1-4, 1 being when the bees were in the centre and 4 being the furthest point out. Each score was then entered into Excel, where averages were calculated, and the data was visualised.

Molecular Work:
Branching off of behavioural work conducted, Real Time qPCR was also used in order to investigate gene expression using RNA extracted from the antenna of honeybees exposed to sulfoxaflor.

RNA Extraction
To extract RNA from antenna, bees were chilled and then antenna removed. To conserve RNA, antennae were immediately placed into tubes and then into liquid nitrogen (so that they were immediately frozen). Once all antennae were collected, the samples were placed in a -80°C freezer to prevent RNA degradation further. RNA was extracted using a commercial kit, following manufacturer's instructions (Zymo Direct-zol RNA purification kits).

Following this, concentrations of RNA were measured using a Nanodrop spectrophotometer.

cDNA Synthesis (reverse transcription)
Reverse transcriptase was used to transcript. mRNA is converted into cDNA. Quantitative PCR requires cDNA, instead of RNA, so that primers will not bind.

Real-Time Quantitative PCR
This process was performed using the cDNA created. The purpose of performing qPCR was to determine whether Sulfoxaflor affected expression of the genes Dop1 & Dop3, which are involved in the development of bees (Vergoz et al., 2009) as they are associated with response to dopamine as a response to QMP.

Data Analysis:

For each set of results, I had to determine the statistical significance. I did this in various ways depending on which test suited the data. For example, for a Mann Whitney U test I used an online software, whereas for a Paired-T Test I used tools provided on Excel.

Because I only had 6 weeks to conduct my research, it meant that sample sizes were quite small. When smaller data sets are used to test for significance, there is often greater chance that they will not be determined as significant. To try to overcome this I managed to

combine related data sets. Many results that I did collect, unfortunately, were not confirmed to be significant. However, my supervisor emphasised that it was not essential for them to be significant. Even non-significant results still provide other researchers with valuable information that can be used in further studies. The work that I completed will be followed up by a new PhD student in Elizabeth Duncan's lab.

References:

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The Personal Impact of my Research

Throughout the period of working in the lab, I developed significantly as a person. I now feel noticeably more confident in myself and my ideas, allowing me to speak out more freely. This occurred as I realised the responsibility that fell upon after me by taking on this project. When I began, I thought that my supervisor was there to tell me exactly what to do and answer every question I had. I soon learnt that this opinion was wrong, my supervisor was

there to initially show me how to carry out experiments and provide guidance, but the main body of work was for me to work through, even when I had uncertainty.

Something that I found helpful was when I approached my supervisor with a query, and she asked me a question back, or directed me to an article that may answer my question. Although this seemed scary at the times, taking this longer route was key in my personal growth. I realised that I have the ability to provide answers to my own questions, as long as I put in the effort to look in the right places.

This time was also the first experience I had of working 9-5 continuously. This proved to be tiring, even though it was only for 6 weeks. Through becoming tired and having to look for ways to motivate myself, I learnt the importance of scheduling my time. This prevented me from getting overwhelmed about work needing to be done, and also allowed me to set boundaries about how much work I'd do in a day so that some time could be kept for myself. Doing this meant that I could avoid burnout and work to my best ability up until the end of my project.

Leadership Skills Gained from the Research Period

At first glance, I wondered if I could develop any leadership skills from my research period as I was mainly working alone. However, I now see that this is not true. Although I was working on my project alone (most of the time), I was always surrounded by my supervisor and her PHD students. This meant that I had people to ask for assistance and somebody to chat to, as days in the lab can be long and quite lonely. This area required the leadership skills involved in developing suitable work relationships. I found that it was sometimes difficult to maintain a balance between being friends and colleagues. Whilst I also had to ensure that I didn't put myself down or feel worried that I was not as qualified as those surrounding me. In addition to this, I had to avoid treating my supervisor as a teacher who would be there to provide constant guidance. To avoid this, I made sure to work through a question before proposing it to Liz. By doing this, I often found that I had to make my own decisions by reading through research papers relevant to the topic in question.

On some occasions, things in the lab did go wrong. Through this, I learnt a good leader should be able to stay calm and swiftly adapt to change. This had to be done a few times throughout the 6 weeks, when my plans for the day were disrupted by fire alarms or bees escaping within the incubator. I learnt to deal with the unexpected situation, then return to what was interrupted and think carefully about whether the process could be continued or if it should be restarted. Furthermore, some things in the lab went wrong due to miscommunication. Because of this, my supervisor and I realised the importance of communication to keep processes running smoothly, using 'Slack' (a messaging app) and by scheduling meetings more frequently.

Preparation is a vital skill needed to mitigate unexpected situations. I kept a suitable level of preparation by keeping a record of everything I did in my lab book, so that I could easily return and understand what had been done, removing any guess work or uncertainty. As well as this, I organised my time with help from my supervisor using a planner, so I could

start each day knowing exactly what needed to be done and plan my time accordingly. I will continue to use these skills and thought processes as I advance in my future work.

Future career, educational plans or continued research plans…

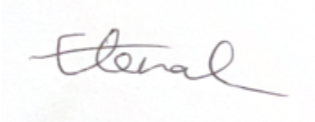
I have thoroughly enjoyed these past 6 weeks of research. It has allowed me to broaden my lab skills into areas that I never would have been able to access without the programme. This has made me certain that I must continue reaching for opportunities that I feel drawn to, even if it's not something that I would usually go for.

Additionally, when I moved from behavioural to molecular work and RNA analysis, I realised how much I enjoy this area biology (using DNA sequencing and genetic information to learn more about organisms). This made me excited to get back to my second year of university and know that I definitely want to continue researching, using biotechnology, in the future.

The significant amount of time that I spent in the lab independently has also given me confidence that I have the ability to work on my own. This has consolidated my decision to apply for a 2024 placement year within the pharmaceutical industry, which I am really looking forward to. I feel like my Laidlaw experience as a whole has already provided me with so many skills that I hope will help me to receive a place at one of my desired companies and help me to achieve even greater things in the future.

Supervisor

Elena exceeded my expectations during this placement. She, very early on, displayed a level of independence that goes beyond what I would expect from third year students. With minimal levels of guidance she established the sucrose assay in my lab. She worked well with the rest of the team and demonstrated her high level of communication skills. These excellent communication skills and tenacity when dealing with situations that went wrong are skills that will stand her in good stead as a leader. Her practical skills are at a very high level. In terms of areas to improve, I struggle to really identify any for Elena. Her critical analysis and troubleshooting abilities will be developed through the rest of her degree, but I noticed significant development of these through the placement. She also became much more independent through the course of her placement. Elena will benefit from more practice in scientific writing, but will get plenty of opportunity to do this throughout the rest of her degree. Having Elena in the lab was a real pleasure and on top of it the data that she generated and the techniques she established will be utilised by a new PhD student in my lab – and Elena will be included as an author on any publication that arises from this. A thoroughly positive experience from my perspective.

Signature of Scholar  Date: 07/12/23

Signature of Project Leader  Date: 20/11/23