The Impacts of Glyphosate on Bumble Bee Productivity and Parasite Load

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A thesis submitted for the degree of

Master of Science

2019

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ACKNOWLEDGEMENTS

First, I would like thank Dr. Neil Reid and Dr. Lorraine Scott for their help and guidance throughout each stage of my research. My thanks to Dr. Lorraine Scott and her husband for their help in sample collection.

To Paul Wallace, for granting me access to many secret gardens hidden around Queen's grounds and for the cheap lavender! To Dan Clarke for allowing me to use the Lock Keeper's Inn for my wild bee observations and for your advice and support.

A huge thank you to those that financially supported my research, because without your kind and generous contributions this research would not have been possible. I appreciate all of your pledges and I will keep an eye out for research that strikes my interest so I can pay it forward.

To my family and friends for listening to me rant. To my mum Mary and my sister Catherine for your encouragement, my dad Desie and William Cooke for your help in building equipment. Thanks to Michaela Burke, Chris McEwan, Nicole Morrow and Niall Greene for helping me collect samples and Robert Stewart for being someone to call on for help and guidance with my research and life.

And to Joshua Toan - for dealing with the good and the bad days, the stressful days, the tears, the nightmares and restless nights. This process has been a rollercoaster and you have been my constant, telling me you believe in me and that it will all work out in the end.

Finally, I would like to dedicate this to my late grandfather Daniel McGurk, without your encouragement growing up, and teaching me that hard work is the secret ingredient to getting everything you dream of, I'm not sure I would have had enough will power to get through my studies and push myself further. I work hard to make you proud, this one's for you Dan the Man. Thank you!

Ps. Thank you to my bees for only stinging me once and for giving me my very first bee sting, how very kind!

Thank You!

ABSTRACT

Pollination services provided by bees play a vital role in sustaining human life, where they can be seen to pollinate just less than 90% of angiosperms and ¾ of agricultural crops. However, there is growing concern over their declining population and the fate of global food security. Studies in the past have identified drivers behind pollinator losses, which include exposure to pesticides such as neonicotinoid insecticides and sulfoxaflor and bee disease. Here, we investigate the impacts of field realistic concentrations of glyphosate on bumble bee health and function. This study used a combination of commercial and wild bumble bee observations, productivity measures and lab analysis to identify the impacts of glyphosate on bumble bee foraging ability and behaviour, productivity and parasite vulnerability. Results obtained suggests that those exposed to glyphosate present hyperactivity or altered flight ability and endurance. Wild bees avoided foraging on treated lavender and visitation to untreated lavender increased with time from glyphosate application, and those fed on glyphosate-laced sugar solution were more vulnerable to pathogen infestation. Such results can potentially have devastating effects on bee mortality, leading to further declines, which may have an impact on crop quality and threaten global food security.

1. INTRODUCTION

1.1 ECONOMIC IMPORTANCE OF POLLINATORS

Pollinator species, including bees play a major role in sustaining human food supplies through the ecosystem services they provide. So much so that they are considered a keystone species, playing a major role in the pollination of just less than 90% of angiosperms and three quarters of agricultural crops, which contribute as much as 35% of global food production (Ollerton, Winfree & Tarrant 2011; Klein et al. 2007). Wild bees in particular are seen to contribute at least 20% of all pollination services within agricultural production (Losey & Vaughan 2006). The extent of pollinator contribution to the human food chain has been evaluated in the past. Animal mediated pollination, which is mostly carried out by bees has a global value of €153 billion per annum (pa) (Gallai et al. 2009), £603 million pa in the United Kingdom and €53 million pa in the Republic of Ireland. (Hanley, Ellis & Breeze 2013; Bullock 2008).

However, bees do more than pollinate crops for human consumption. Pollinators more specifically honey bees are important contributors to modern medicine. Many by-products of honey bee colonies possess numerous health benefits, for example, honey possesses anti-bacterial properties (Cooper & Molan 1999) and has been used in wound dressings in the United States since 2007. Manuka honey (MH) which is produced in New Zealand and Australia, is a monofloral honey that is collected from *Leptospermum scoparium* tree. MH has often been used in traditional medicine (Mandal & Mandal 2011), but possesses many applications due to its antioxidant, anti-inflammatory, immune-modulatory, antibacterial and wound healing properties (Afrin et al. 2018a, b; Morroni et al. 2018).

Therefore, it can be assumed that there would be detrimental consequences of pollinator losses to human life and well-being. In fact, a study by Smith et al investigated the potential impacts that a complete loss of pollinators would have on human life. The 2015 study found that 71 million people, living in low income countries would become newly deficient in vitamin A, as well as 2.2 billion (whom are currently consuming less vitamin A than they require) experiencing further declines in vitamin A supplies. Additionally, there would be 22.9%, 16.3% and 22.1% reductions in the global production of fruit, vegetables and nuts, respectively. This would bring an increase of 1.42 million or 2.7% in total annual deaths (Smith et al. 2015).

1.2 POLLINATOR DECLINES

There is approximately 20,000 different species of bee worldwide (Richardson et al. 2019). However, pollinator species like bees have in the past and are continuing to experience declines in their population numbers and biodiversity (Allen-Wardell et al. 1998; Steffan-Dewenter et al. 2005). It is said we are currently undergoing a 'pollinator crisis' where both managed and wild pollinators are suffering losses, threatening global food security, and it is believed that such declines are a product of human activity (Bartomeus et al. 2018; Holden 2006; Westerkamp & Gottsberger, 2002).

Anthropogenically driven effects habitat destruction and degradation and chemical intensive agricultural practices are considered key drivers behind pollinator losses. As these drivers continue, important pollinator species like bees are forced to adapt or face the threat of extinction (Zayed 2009). Since 1980 more than half of Ireland's bee species have suffered major declines in their population numbers and as many as 30% of all bee species in Ireland are threatened with extinction. (Fitzpatrick et al. 2006a). Three have already become extinct in the last 80 years (Fitzpatrick et al. 2006b).

The topic of pollinator losses and declines has become one of the highest profile global environmental issues of the 21st century (Brown et al. 2016), and the growing concern of their declining population has been a popular discussion between governmental bodies across the globe. There is need for cohesive and research led, national policies which look at past and current threats to pollinator population (Brown et al. 2016) for their conservation.

Many farmers and stakeholders worldwide are concerned about the declining pollinator population as they depend upon their pollination services for their cultivated crops. Many believe that wild pollinators can no longer provide adequate pollination services which is required for crop production (Allen-Wardell et al, 1998; Steffan-Dewenter et al. 2005), thus potentially threatening their crop yield potential and with it, their livelihood. To combat this, more and more growers and farmers are opting for the use of commercially reared honey and bumble bees to provide the pollination services which they require (Lye et al. 2011). Commercially reared bumble bees have been available for pollination supplementation for just over 30 years. In fact, it was Dr Roland De Jonghe who first introduced the practice, founding the company Biobest in 1987 and to this day Biobest remains one of the largest producers of commercial bumblebees (Goulson, 2014). In the United Kingdom approximately 30 – 60,000 bumble bee colonies are imported each year, for their use in pollination supplementation (Lye

et al. 2011). However, evidence shows that wild pollinators continue to contribute substantially to crop production, especially in areas of low to moderate agricultural intensity. It is areas like these that provide wild bees with a mosaic of crops in close proximity to natural or semi-natural habitats that provide bees with nest sites allowing them to thrive (Ricketts et al. 2008; Klein et al. 2007; Kremen et al. 2004). Yet, more land is being utilised for the cultivation of high-value pollinator-dependent crops, with no consideration of its impacts upon wild pollinator numbers. In fact, in the last 5 decades there has been approximately a 25% expansion in cultivated area by global agriculture, most of which has been taken up by pollinator-dependent crops (Aizen et al. 2008). There is growing concern that global commercial pollinator stocks are not sufficient to sustain the pollination demand, especially if more land is being transformed for the cultivation of pollinator-dependent crops (Aizen & Harder 2009). If this is the case, growers across the globe may depend more heavily on the use of synthetic chemicals to improve their crop yields and crop quality, thus bringing major increases in chemical intensive agriculture practices. Which also has been shown to have negative effects on wild pollinators (Evans et al. 2018; Mancini, Woodcock & Isaac 2019).

1.3 CHEMICAL INTENSIVE AGRICULTURE

The current global population stands at 7.7 billion and continues to grow. In July 2019 the United Nations issued a report that outlined an expected increase of 2 billion persons by 2050, bringing the global population to 9.7 billion in just 30 years (United Nations 2019). The ever-growing population has led to concern over global food security. To combat this, many growers have turned to the use of chemical-intensive agriculture practices to increase crop yield (Siviter, Brown & Leadbeater 2018), prevent disease and to protect crops from pests and competing flora (Shaw & Arnold 2002). With increased chemical intensive agricultural practices, bee pollinators are likely to face increased habitat loss and degradation, reduction in floral resources and exposure to the chemicals themselves, which they must overcome to survive (Kenna et al. 2019). This is concerning due to the significant amount of human food supply that is reliant upon the ecosystem services provided by insect pollinators.

The introduction of new technology, powerful machinery and synthetic pesticides marked the beginning of the industrial agriculture era. The growing population threatened global food production leading to increased use of pesticides (herbicides, insecticides and fungicides) and fertilisers to increase crop yield and quality (Aktar, Sengupta & Chowdhury

2009). Pesticides in the past have provided many global benefits, not only to global crop production but also to human health. More specifically pesticides have helped improve crop quality and productivity as well as protecting crops against competing weeds, insect pests and disease. They have also played a role in disease control in humans, for example mosquito control to prevent the spread of malaria (Aktar, Sengupta & Chowdhury 2009).

The changes associated with the movement to modern agriculture were a cause of concern for the health of the environment, biodiversity and human beings. In today's society, as much as 3 million tons of pesticide is used per year (Horrigan, Lawrence & Walker 2002). However, growing evidence supports the view that pesticides pose potential threat to human health and well-being and undesirable impacts on the environment and non-target organisms i.e. pollinating insects (Forget 1993; Giedion 1991; Jeyaratnam 1981). In fact, insecticides have been directly linked to the death of bees and other insect pollinators, and herbicides indirectly cause declines in bee populations by reducing the diversity of their feeding resources (Sánchez-Bayo et al. 2016).

A pesticide which has often made headlines for their impacts on bees, is a group of insecticides called neonicotinoids. Neonicotinoids are the most widely used class of insecticides and have been a part of agricultural practices since the late 1990s (Van der Sluijs et al. 2013). They are used to control herbivorous insect pests. However, because only 1.6-20% of the substance is absorbed by the crop, non-target organisms are at risk of coming into contact with the remaining substance while foraging (Sur & Stork 2003). This is particularly dangerous for bees as neonicotinoids interact with the nicotinic acetylcholine receptors (nAChRs) in their central nervous system. This can lead to neuronal hyperexcitation, paralysis or even death in the bees (Belzunces, Tchamitchian & Brunet 2012; Tomizawa & Casida 2005). A study carried out in 2007 found that bees exposed to sub lethal doses of neonicotinoids presented changes in their feeding behaviour, navigation orientation, immunology, sex ratio and learning ability (Desneux, Decourtye & Delpuech 2007), all of which had colony, population and community level impacts. Such discoveries led to a moratorium of three seed coatings (clothianidin, imidacloprid and thiamethoxam) in 2013 and a later a Europe-wide ban of neonicotinoids in 2018 (Gross 2013).

It is a cause for concern that other frequently used pesticides i.e. herbicides, particularly those containing glyphosate, can potentially have impacts as devastating as those of neonicotinoids on insect pollinators.

1.4 GLYPHOSATE BASED HERBICIDES

Glyphosate, which is the active ingredient found in herbicides such as Roundup, was first discovered in the 1950s by Henri Martin, but its potential as a weed-killer was not recognised until the early 1970s. It was first approved and released to the commercial market by Monsanto in 1974 (Morini, Frank & Fenner-Crisp, 2018; Schmitz and Garvet 2012). Glyphosate inhibits the enzyme 5-Enylpyruvylshikimate-3-phosphate synthase (EPSPS) found in the shikimate pathway, which is only present in plants and some microorganisms (Dill 2005). It works by preventing the biosynthesis of aromatic amino acids and secondary metabolites (Motta, Raymann and Moran, 2018).

Over time Glyphosate became the most widely used herbicide across the globe. Its widespread applications brought changes to the agricultural industry with its ability to effectively control weeds, and it remains the backbone of modern weed management (Green 2016). Although the primary purpose of glyphosate was to control weeds, it was often used as an agronomical instrument (Steinmann, Dickeduisberg & Theuvsen 2012). Farmers and growers often used glyphosate in pre-sowing, to reduce tillage, pre-seeding, to eradicate annual weeds before the establishment of a crop (Beckie 2006) and pre-harvest, to dry the crop allowing for easier harvest (Cook, Wynn & Clarke 2010; Morini, Frank & Fenner-Crisp 2018), all of which saved labour and machinery costs.

In the beginning glyphosate was used only in non-crop and orchard production systems as it destroyed vegetation upon application. However, with time and the development of new technology, agricultural crops were soon able to be genetically modified making them more tolerant to glyphosate. This was done by inserting a gene taken from a bacterium called *Agrobacteria* into various agricultural crops genetics, thus creating Roundup Ready crops (Padgette et al. 1995) and marking the beginning of the herbicide-resistant crop revolution (Green 2016). However, before the introduction of herbicide resistant (HR) crops, the application of glyphosate was limited to soil application prior to planting or directed spraying. The development of HR crops made it easier for farmers and growers to control weed growth and allowed them to achieve higher and more profitable yields (Brookes & Barfoot).

For 35 years glyphosate-based herbicides have been utilised in weed management practices (Duke & Powles 2008) and for 15 years it was the sole method of weed control (Green 2016). However, the movement from ‘traditional’ use of glyphosate to its current multi-application herbicide, has led to concern that glyphosate-resistant weeds may become the norm

(Duke & Powles, 2008a). In fact, it is believed that the increasing usage of glyphosate will encourage the evolution of glyphosate-resistance, thus leading to further concern that growers will, instead of diversifying their weed management practices, use higher concentrations of glyphosate in combination with other synthetic chemicals in efforts to control weeds (Dill 2005).

In recent years there has been growing concern regarding the use of glyphosate-based herbicides and how they impact human health, animal health and insect pollinators. It was believed that because humans and animals lack the shikimate pathway, that exposure to glyphosate would not have an impact upon their health (Duke & Powles 2008b). However, recent studies suggest potential links between the consumption of crops treated with glyphosate and various health issues in human beings such as: gastrointestinal disorders, obesity, diabetes, heart disease, depression, autism, infertility, cancer and Alzheimer's (Krüger et al. 2014). Such findings have led to concern over the impacts of glyphosate upon pollinating insects. In fact, a study carried out in 2015 found that bees which were fed sucrose that contained 2.5mg, 5mg, and 10mg l -1 glyphosate experienced changes in their flight ability and trajectories, suggesting that exposure to glyphosate impairs navigation and has an impact on their spatial learning processes (Balbuena et al. 2015). It is therefore important that future research sets out to investigate the impact of both field realistic and sub-lethal, cumulative concentrations of glyphosate on managed and wild bees. Such studies could be used to determine if exposure to glyphosate may be acting as a driver behind declining pollinator populations, allowing for the development of informed management plans for their conservation.

1.5 SUMMARY AND AIMS OF STUDY

This study set out to investigate potential impacts of glyphosate-based herbicides on the health and function in bumble bees, specifically, their foraging activity rate, foraging preference, colony productivity and disease vulnerability.

The main questions this study sets out to answer are listed below;

Does exposure to glyphosate:

1. have an impact on bumble bee foraging activity?
2. make bumble bees more susceptible to contracting parasites and pathogens?
3. have an impact on colony productivity?

It is hypothesised that bumble bees which are exposed to glyphosate-based herbicides will experience

- increased vulnerability to parasite and pathogen infections
- decreased foraging ability
- decreased productivity

2. MATERIALS AND METHODS

2.1 EXTERNAL FUNDING

The high cost of commercial bumblebee colonies meant that external funding was required to cover the cost of the project. Extensive research was carried out to evaluate the most appropriate method of external funding. It was decided that crowdfunding was the most feasible option and a crowdfunding website dedicated to the funding of scientific research called Experiment.com was chosen. To launch a project on the Experiment website a full project overview was required covering what the research was investigating, its importance, budget breakdown, research timeline and a video interview. Once approved the project was promoted using social media platforms such as Twitter, Facebook and Instagram. This project had an overall target of $2000 and the project ran for 30 days.

Figure 1 Screenshot of crowdfunding project on Experiment.com

2.2 PILOT STUDY

A pilot study was carried out from 16th May - 5th June 2019. This involved the use of one test hive which was deployed for a total of 3 weeks, during which hive set up, sample collection, colony weighing, and treatment techniques were carried out to reduce disturbance and stress to the colonies during the main body of research. Treatment was carried out prior to the core research to test the concentration of glyphosate chosen, to ensure it was non-lethal to the bumblebees, preventing death to all experimental colonies.

2.3 EXPERIMENT ONE

Experiment one was carried out from the 6th June – 8th July. A total of 20 Biobest *Bombus terrestris audax* colonies were purchased, which contained 80 live bumble bees on arrival. Supplementary feeders were opened, and colonies were placed in a zig-zag pattern across the edge of the garden. Colonies were set on top of two concrete bricks to prevent flooding or overheating, with one on top to prevent movement.

Due to the vast number of colonies, each colony was assigned a unique code and marker to allow the bumblebees to identify their colony with more ease, as an attempt to prevent drift from colonies. The colonies were then set on the entry and exit setting, allowing the bees to exit their colony to forage.

Samples of bees before treatment were collected over a week period. This was done by waiting for exiting bees and catching them with a net and transferring them to a tube containing ethanol.

Unfortunately, before treatment could be carried out, the colonies showed signs of dysentery (Figure 2) and were no longer fit for their research purposes. Colonies were then packed and frozen. Further samples were taken after freezing and all colonies were disposed of appropriately.

Figure 2 Photograph showing the presence of dysentery on the front of one of the colonies used in experiment 1

2.4 SITE SELECTION

A total of three research sites were used throughout the study, each site was assigned a specific experimental role to cover each aspect of the research question. The wildlife garden located beside Queen's McClay Library was used for experiment one, Lennoxvale Lake was used for experiment two which involved the release of commercially bought *Bombus terrestris* and The Lock Keeper's Inn at Lagan Valley Regional Park was used to perform choice experiments on wild bumblebees.

The Wildlife Garden

The wildlife garden (Figure 3) was used to perform experiment one. This site was chosen specifically for its location – adjacent to Belfast's Botanical Gardens, which was believed to provide the commercial bees with an abundance of foraging resources, as well as being close to the school of Biological Sciences – where all subsequent laboratory analysis took place. The site being in close proximity to the school also meant that the commercial bees were subject to less stress during transport following their delivery. The wildlife garden is private and is only accessible with prior authorisation. This meant that the site's traffic was low, thus greatly reducing the rate of human disturbance, damage or vandalism.

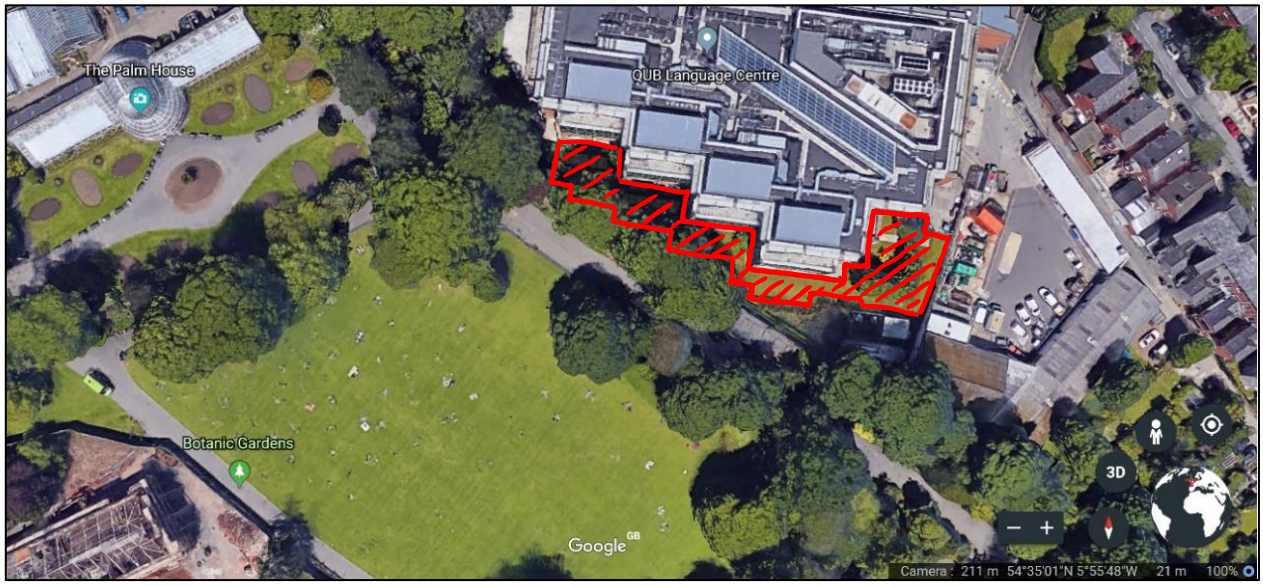

Figure 3 Satellite image of the wildlife garden at the McClay library (highlighted in red)

Lennoxvale Lake

Lennoxvale Lake (Figure 4) was used for the core experimental project (experiment two). This involved the use of two of Koppert's Tripol hives to investigate the effects of glyphosate on: colony productivity, foraging behaviour and parasite burden. This site was selected as it provided an abundance of floral resources which the commercial bees could utilise for foraging, ample space so that the hives could be placed approximately 100m apart, to prevent drift from treated and control colonies and plenty of tree cover to prevent the colonies from overheating. This site can only be accessed by a locked gate, therefore once the colonies were placed and deployed, they did not experience any unnecessary human disturbance. This site, like the Wildlife Garden, lay within close proximity to the School of Biological Sciences which meant that following the delivery of the hives the commercial bees were not subject to added stress which they would experience during travel.

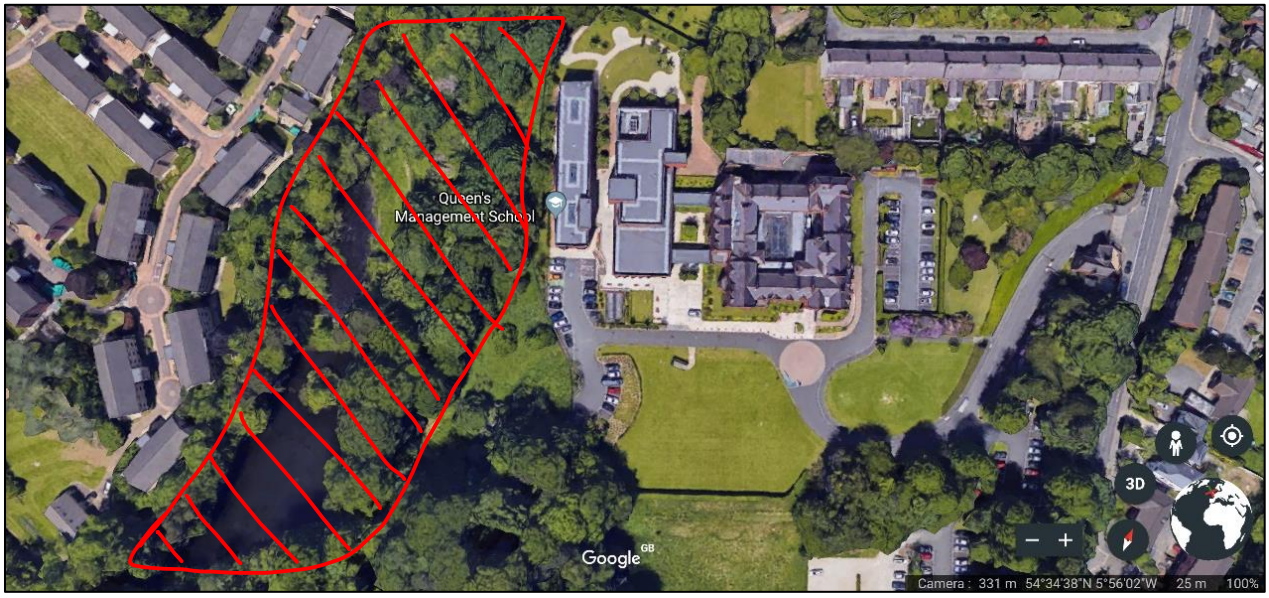

Figure 4 Satellite image of the site at Lennoxvale Lake (highlighted in red) used to deploy commercial colonies

The Lock Keeper's Inn at Lagan Valley Regional Park

The Lock Keeper's Inn site (Figure 5) was used to perform the wild pollinator choice experiments. This section of the research used treated and non-treated lavender to investigate bumblebee foraging preferences to measure their productivity and behaviour. When visiting the area around the Lock Keeper's Inn, prior to carrying out the research, it was evident that this site was popular for foraging bumblebees. The nearby cottage provides visiting bees and other pollinators with foraging resources. It was therefore thought that this site would be suitable for the wild pollinator observations as pollinator presence was already apparent and would reduce the time spent encouraging bees to visit the lavender.

Figure 5 Satellite image of the Lock Keeper's Inn at Lagan Valley Regional Park, where wild bee observations were carried out (highlighted in red)

2.5 WILD OBSERVATIONS

A total of 80 lavender plants were purchased which were close to full bloom on arrival. Lavender was kept and maintained at home until it was suitable to use for visitation observations. Each observation series utilised 16 plants. Plants which were in full bloom were selected and placed in two trays of eight. One tray was treated at home by spraying ready-to-use Roundup, which contains 7.2g/l glyphosate acid (present as 9.7g/l) IPA salt of glyphosate in a ready to use solution, while carrying out the required safety precautions. The plants were then transported to the Lock Keeper's Inn in the boot of a car.

Once at the Lock Keeper's Inn the treated and non-treated lavender were arranged in a random pattern in a 4X4 grid (Figure 6) and separated by sheets of white paper to allow each plant to be distinguished easily.

Figure 6 Random arrangements used and photographs of test lavender at 1hr, 24hrs and 48hr for each replicate

A total of 5 replicates were carried out over the course of 4 weeks. Each replicate was made up of 3 days which consisted of a 1-hour observation at; 1 hour, 24 hours and 48 hours after treatment (total of 3 hours observation per replicate). Each observation was completed in 15-minute increments (15 minutes observation, 15 minutes rest) and was run for a total of two hours from 10:15am-12:15pm.

Observations were run in 15-minute increments to prevent human error and inaccuracy due to fatigue. During observations, plant visitation by bumble bees only were formally recorded, however additional notes of other pollinators such as honey bees and hoverflies were recorded.

Plants were stored in a locked area to prevent damage, vandalism and theft, and to reduce potential contact with the public due to the application of glyphosate. At the end of each replicate the plants were removed and disposed of appropriately.

2.6 EXPERIMENT TWO

A total of two Koppert Tripol hives (each containing 3 colonies) of bumble bee species *Bombus terrestris* were purchased, with each hive made up of approximately 350-400 workers, brood and a queen. Once the hives were delivered on the 25th July 2019, they were transported to Lennoxvale Lake and placed in the field approximately 100 meters apart, to prevent drift from one hive to the other. Hives were placed in a shaded area and were set on top of two concrete bricks to prevent flooding and overheating with a brick on top to prevent movement.

Each hive was assigned a treatment group; treated – glyphosate spiked sugar solution, and a non-treated control. Each colony (n=6) was allocated a unique identification code which was used to keep record of productivity and activity measurements taken throughout release period.

Weight measurements were taken on a weekly basis using a digital weight hook. Successful measurements were taken on day 1, week 1 and week 3. Weight measurements were not able to be recorded due to an amber weather warning at week 2. The weather meant that the site was no longer safe and visiting the site in such conditions would have gone against health and safety regulations. A field realistic concentration of 3.7mg/l glyphosate was used (Herbert et al., 2014; Giesy et al., 2000). Ready to use Roundup was used as the source of glyphosate, which contains 7.2g/l glyphosate acid (present as 9.7g/l) IPA salt of glyphosate in a ready to use solution, and therefore required further dilution. It was calculated that 0.5139ml of the

ready to go Roundup solution was added per litre of sugar solution to give an overall concentration of 3.7mg/l glyphosate.

See calculation below:

$$\frac{7.2\text{g/L}}{3.7\text{mg/L}} = \text{a dilution factor of}$$

$$\therefore 1\text{L of Roundup}^{®} / 1945.9 = 0.5139$$

The amount of sugar solution present within the supplementary feeders was measured to determine the amount of roundup required to achieve the desired concentration. The bees were locked in their hives for 24 hours to ensure consumption of treatment and were opened the following day. The supplementary feeders remained accessible for the whole 3-week experimental period.

The volume of sugar syrup was recorded at the beginning (day 1) of the experiment and again after the 3-week release period. This was done to determine the total volume of sugar solution consumed over a 3-week period by each of the colonies.

A total of 6 worker samples were collected from each colony. Samples were collected by intercepting bees which were exiting the hive. Samples were stored in ethanol, labelled and transported to the lab for subsequent analysis. Hives were then left on the entry and exit setting for 1 week.

Behaviour observations were carried out at 1 week, 2 weeks and 3 weeks. Observations were run between 10am-1:30pm, where entry and exit from each colony was recorded over a 30-minute period, this was repeated 3 times for each hive, making up a total of 1.5 hours observation per hive. Following observations each hive was set on the entry only setting, allowing foraging bumble bees to return to their colonies, but preventing others from exiting. This was done to allow colony weight and sample collection to be carried out that night at approximately 6pm. At this time all foraging bees would have returned to their colonies and would be settled to rest until the morning. Samples were collected by opening the top of the colonies and catching flying bees in a net or simply by picking them out from the top of the nest (taking caution not to take a queen) and samples were stored in ethanol.

Following the last replicate of commercial behaviour observations, the hives were set on entry only setting and left over the weekend to ensure all foraging bees returned to their hives. On Monday 19th August, colonies were collected and transported to the school of Biological Sciences where they were frozen for 24 hours.

2.7 Lab Analysis

Productivity

Colonies were removed from the freezer and allowed to defrost. Once thawed each colony was weighed and the volume of sugar solution remaining in the supplementary feeders was recorded. The total number of bees (which were later sorted into castes – queens, workers and drones) were counted and the number of capped brood cells easily seen on the surface of the nest were recorded. All colonies and remaining sugar syrup were disposed of in the appropriate manner.

Parasitology

External Parasites

External parasites were recorded by viewing specimen samples under a microscope (Figure 7). In cases where the commensal parasite *Parasitellus fucorum* were found in a pooled sample, groups were marked as yes, and in the absence, they were marked as no.

The number of commensals infesting bees within a sample were not counted as their presence did not impact negatively on the individual or the colony as a whole.

Figure 7 Microscope image of Bombus terrestris sample and commensal parasite *Parasitellus fucorum*

Testing for *Nosema*

A total of 6 specimens per samples were collected from each colony (3 glyphosate treated and 3 controls) on day 1, week 1 and week 3 to test for the presence of *Nosema ceranae* spores and the level of infection. The tissues within the abdomen (ventriculus, venom sack, crop etc.) were dissected out to reduce the amount of debris. It should be noted that dissection equipment was sterilised with alcohol between samples. Dissected tissues were diluted with 3ml deionised water and mixed using a micro pestle and mortar. The samples were pipetted into centrifuge tubes. Samples were mixed using a vortex to ensure spores were evenly distributed. Using disposable pipettes, a small drop of the samples were placed on a microscope slide and left to dry. When dry slides were view under a light microscope and X40 magnification. *Nosema ceranae* spores were identified by looking at their shape size and reflectivity as stated in MacFarlane and Larsson (Mcfarlane et al. 1995; Larsson 2007).

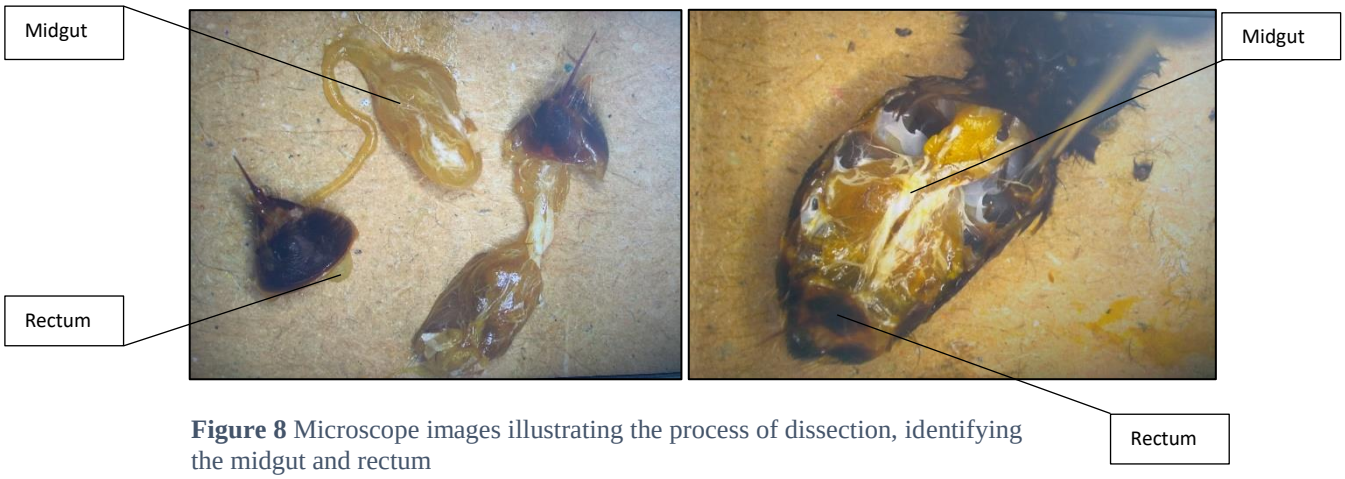

Figure 8 Microscope images illustrating the process of dissection, identifying the midgut and rectum

Spore which were present within a 1mm by 1mm grid were counted. Only spores entirely present within the square and those located on the bottom and right borders were counted to prevent spores from being counted more than once. This process was repeated twice for each sample.

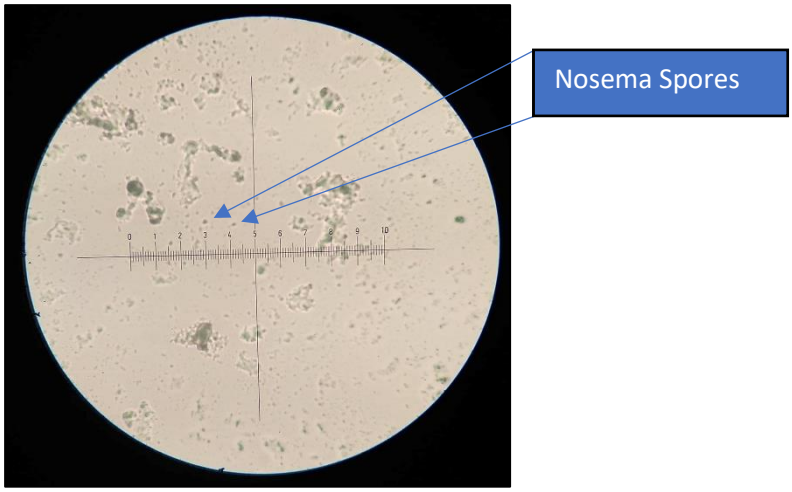

Figure 9 Light microscope image viewing *Nosema ceranae* spores at x40 magnification

The total number of particles per ml was calculated using the formula below (Human et al.);

$$\frac{\text{Total number of counted particles x dilution factor}}{\text{Area of squares counted (mm}^2\text{)}} = \text{items per ml}$$

and the total number of particles present within a sample was calculated by multiplying the concentration obtained by the initial sample volume.

2.8 STATISTICAL ANALYSIS

The initial failure of 20 colonies set up for experiment during June 2019 was investigated by examining variation in disease status (infection by *N. ceranae*) and weather conditions. Median parasite load was compared with replacement colonies (July/Aug 2019). Minimum and maximum air temperature and rainfall during June 2019 was compared to the historical recent average (2009-2018) using t-tests.

Replacement bumblebee colony activity (the number of individual bees entering or exiting the colony) per half hour focal observation per week was examined using a Generalised Linear Mixed Model (GLMM) where wither entrances or exits were fitted as the Dependent variable and Week number (1,2,3) and Treatment (Glyphosate or Control) were fitted as Independent factors. The observation bout (each half hour observation period) was fitted as a Random Factor to account for multiple observations per colony (i.e. pseudoreplication).

Bumblebee visitation rates to glyphosate treated lavender plants versus non-treated controls was examined also using GLMM where Treatment (Glyphosate or Control) and Time since spraying (1hr, 24hr and 48hrs after treatment) were fitted as Fixed Factors. Five replicate observations were taken with replicate fitted as a Random Factor to account for multiple observations per treatment (i.e. pseudoreplication).

3. RESULTS

A total of 20 colonies (10 glyphosate treated and 10 controls) were initially set up but all showed signs of dysentery infection. Upon examination, pooled samples from these colonies had a concentration of *N. ceranae* spores of 3.0 spores/ml which was similar to that of replacement colonies (3.5 spores/ml in untreated controls verses 2.5 spores/ml in glyphosate treated colonies). However, weather conditions during June 2019 were historically atypical having significantly lower minimum air temperatures (1ºC cool than average; *t*-test = 3.686, $p<0.001$) and significantly more rainfall (146% more; *t*-test = -4.194, $p<0.001$) than the average from 2009-2018 though maximum air temperatures were similar (*t*-test = 1.433, $p=0.153$; Table 1). Thus, it seems likely any dysentery may have been a consequence of being restricted within the colony due to unfavourable foraging conditions forcing bees to consume their solid food stores which can present as diarrhoea.

Table 1 Annual weather conditions (minimum and maximum air temperatures and rainfall) for the decade before June 2019 compared to conditions during June 2019 (initial failed experiment).

Period	Year	Min Temp °C	Max Temp °C	Total Rainfall (mm)
Previous decade	2009	1	23	46.3
	2010	7	21	33.3
	2011	5	18	65.1
	2012	3	19	80.1
	2013	6	21	31.8
	2014	6	20	27.7
	2015	3	22	42.5
	2016	6	22	43.3
	2017	7	22	50.8
	2018	7	24	22.2
	MEAN	**5.1**	**21.2**	**44.3**
Experimental year	**2019**	**4**	**21**	**109.0**

3.1 Does Exposure to Glyphosate Affect Bumblebee Foraging Activity?

The number of bumblebees entering and exiting their colonies decreased significantly from Week 1 to Week 3 with glyphosate treated colonies being significantly more active than control colonies i.e. more bees entering and exiting per unit time (Table 2; Figure 10).

Table 2 Generalised Linear Mixed Model (GLMM) for the number of bumblebees entering colonies during half hour focal observations.

a) Bumblebee entrances

Source	F	df1	df2	Sig.
Corrected Model ▼	5.952	5	47	.000
Week	11.359	2	47	.000
Treatment	5.587	1	47	.022
Week*Treatment	0.642	2	47	.531

Probability distribution: Gamma
Link function: Log

b) Bumblebee exits

Source	F	df1	df2	Sig.
Corrected Model ▼	7.175	5	47	.000
Week	12.775	2	47	.000
Treatment	8.034	1	47	.007
Week*Treatment	1.050	2	47	.358

Probability distribution: Gamma
Link function: Log

Bee entrances **Bee exits**

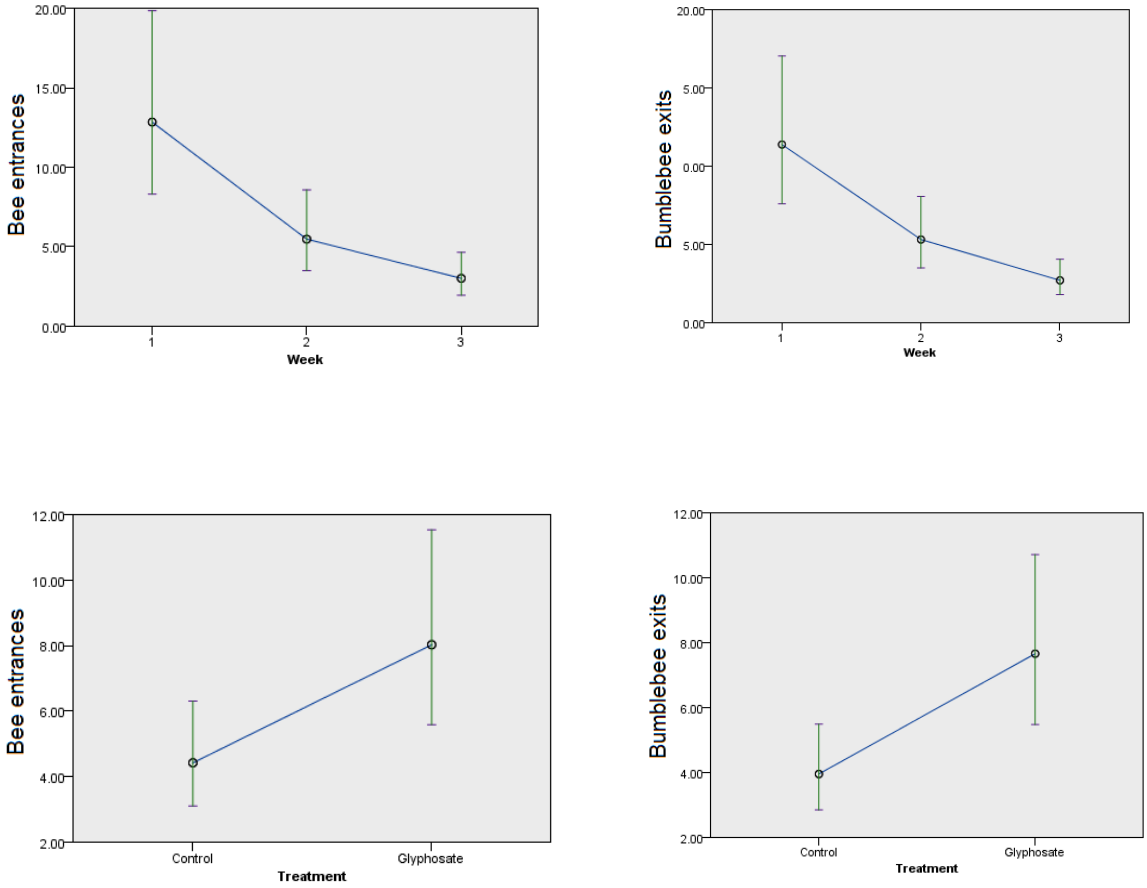

Figure 10 Bumblebee activity (numbers entering or exiting the colony) across experimental weeks (top panel) and comparing glyphosate treatment to controls (bottom panel).

Free living bumblebee visitation to a 4x4 grid of 16 lavender plants (half sprayed with glyphosate randomly and half untreated controls) suggested that visitation rates to treated plants was significantly lower than control plants (Table 3) even after 1-hour post-spraying with the effect persisting for up to 48 hours (during which plants visibly began to die back). Whilst there was no significant effect of time (between 1hr, 24hr and 48hrs after treatment) bumblebee visits to control plants marginally increased over the three days of observation (Figure 11).

Table 3 Generalised Linear Mixed Model (GLMM) for the number of bumblebees visiting lavender (Lavandula angustifolia) plants (treated with glyphosate or controls) over three days after spraying (Time).

Source	F	df1	df2	Sig.
Corrected Model ▼	6.222	5	24	.001
Treatment	27.934	1	24	.000
Time	1.078	2	24	.356
Time*Treatment	0.511	2	24	.606

Probability distribution:Gamma
Link function:Log

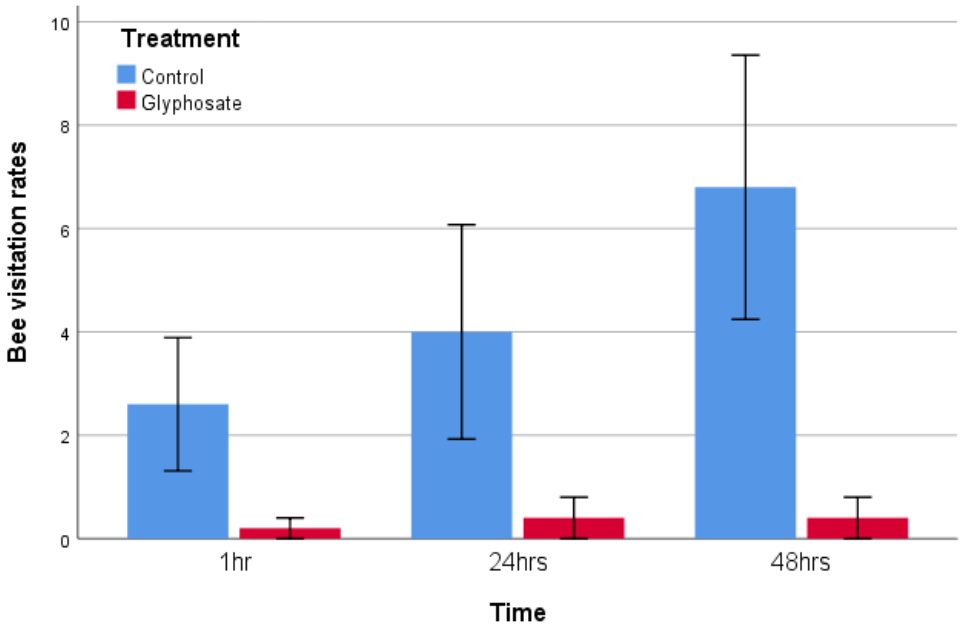

Figure 11 Bumblebee visitation to lavender (Lavandula angustifolia) plants (treated with glyphosate or controls) over three days after spraying (Time).

3.2 DOES EXPOSURE TO GLYPHOSATE MAKE BUMBLEBEES MORE SUSCEPTIBLE TO CONTRACTING PARASITES AND PATHOGENS?

There was no significant effect of either treatment, time or their interaction on the *N. ceranae* spore concentration in bumblebees sampled from glyphosate treated and untreated controls over time (Table 4). It appeared that glyphosate treated bumblebees had increasing concentrations of the parasite over time (higher in Week 1 than Day 1 and highest in Week 3) yet there was no clear pattern over time in untreated controls (Figure 12).

There were only two instances of the bumblebee mite (*Parasitellus fucorum*) infection with both instance occurring on glyphosate treated colonies (11% occurrence); all control bees examined were mite free (0% occurrence).

Table 4 GLMM for the concentration of N. ceranae spores per ml of six pooled bumblebee ventriculi sampled per colony over Time (Day1, Week1 and Week3) with the effect of treatment (treated with glyphosate or controls).

Source	F	df1	df2	Sig.
Corrected Model ▼	1.735	5	12	.201
Treatment	0.005	1	12	.943
Time	2.643	2	12	.112
Treatment*Time	1.692	2	12	.225

Probability distribution:Normal
Link function:Identity

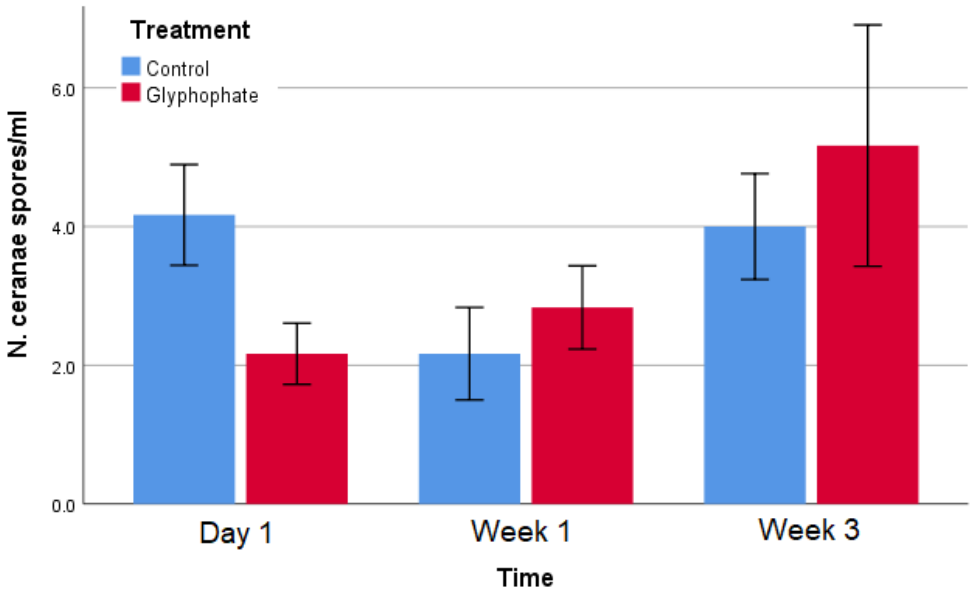

Figure 12 Mean ± 1SE concentration of N. ceranae spores per ml of six pooled bumblebee ventriculi over Time (Day1, Week1 and Week3) with the effect of treatment (treated with glyphosate or controls).

3.3 DOES EXPOSURE TO GLYPHOSATE HAVE ANY EFFECT ON OVERALL BUMBLEBEE COLONY PRODUCTIVITY?

As there were only three replicate colonies in each treatment group (3 glyphosate treated and 3 untreated controls: n=6) no test of difference for any measure of colony productivity showed any statistical difference (Mann Whitney U, p>0.05). Nevertheless, glyphosate treated colonies gained 500% more weight over a three-week period than untreated controls (Table 5). Typically, glyphosate treated colonies had 20% fewer workers and no drones compared to untreated controls. Glyphosate treated colonies had 240% more brood cells indicating higher reproductive effort during the three-week experiment. Correspondingly, glyphosate treated colonies consumed 132% more supplementary sugar solution than untreated controls.

Table 5 Seven measures of bumblebee colony productivity comparing median values for each between glyphosate treated and untreated controls (showing % difference).

	Median values		
Productivity measure	**Untreated controls**	**Glyphosate treated**	**% Difference**
Colony weight	20	120	500
No. of Queens	1	1	0
No. of Workers	74	59	-20
No. of Drones	3	0	-100
No. of individuals	74	60	-19
No. of brood cells	15	51	240
Volume (ml) of supplementary sugar solution consumed	220	510	132

4. DISCUSSION

4.1 EXPERIMENT ONE

Results obtained in experiment one suggests that the clear symptoms of dysentery (Figure 2) was a result of unfavourable weather conditions, as samples from experiment one were found to be no more infected than those sampled in experiment two. Dysentery, although considered a symptom of *Nosema* infections, can also occur in bouts of bad weather, where bees are unable to exit the hive to forage or perform cleansing flights. If bad weather persists and bees are unable to exit the hive to forage, they consume feeding material present in their hive, this often contains solids which are difficult for bees to digest and leads to too much bulk within the intestine. In cases where bees are unable to exit the hive to perform cleansing flights, bees often defecate inside, or just outside of the hive (Honey Bee Suite 2019).

4.2 FORAGING ABILITY

Commercial colonies

The number of entrances and exits from treated colonies was significantly higher than those recorded in control colonies (Figure 10). A possible explanation could be that bees exposed to field-realistic concentrations of glyphosate experienced hyperactivity (Boily et al., 2013), increasing their motivation to exit the hive to forage. In cases where this result is looked at in isolation of the amount of supplementary feed consumed during this study, it could be considered that the increased rate of activity in glyphosate-treated hives is a consequence of individuals avoiding glyphosate laced sugar solution (de Brito Sanchez et al. 2015; Avarguès-Weber et al. 2010; Bermant & Gary, 1966; Rodríguez-Gironés, Trillo and Corcobado 2013). In this instance, this is unlikely as treated colonies were seen to consume 132% more of the supplementary sugar solution than control colonies (Table 5). Therefore, it may be more accurate to assume that exposure to glyphosate causes reductions in bee flight ability, more specifically flight distance and endurance (Kenna et al. 2019; Balbuena et al. 2015), causing them to return to their hive more frequently within a short time period, thus making it appear that glyphosate-treated colonies are more active than that of the controls.

However, a study which investigated the impact of thiamethoxam (a type of neonicotinoid insecticide) on honey bee foraging ability found that acute exposure to thiamethoxam brought about an increase in flight endurance (Tosi et al. 2017). In contrast, a study by Kenna et al., found that bumble bees which were subject to an acute exposure of imidacloprid (a type of neonicotinoid insecticide), experienced reductions to overall flight ability, reducing both flight distance and duration. In fact, this study observed that treated worker's flight ability was a third of what control workers achieved (Kenna et al. 2019). Moreover, it is important to be cognisant of the different study species and chemicals used in these studies when making comparisons to the results of the current study.

It is important to acknowledge that this study looked at foraging activity at colony level, where entrances and exits from the hive only were recorded and did not look at activity carried out while foraging. Therefore, to further investigate the impacts of glyphosate on bumble bee foraging activity and ability, a sample of workers could be radio tagged to allow for the tracking of their daily foraging activity to identify changes in flight patterns. This method was used in 2011 by Decourtye et al., where Radio Frequency Identification (RFID) tags were used to measure the effects of pesticides on honey bees' foraging ability and behaviour (Decourtye et al. 2011).

Wild observations

It is clear from the results obtained during wild observations (Figure 11) that wild bees did not frequently visit glyphosate-treated plants and that there was no significant difference in visitation rate across the 3 days (1hr, 24hrs and 48hrs). This is likely because treated lavender started to die back almost immediately after treatment (Kirkwood et al. 2000; Caseley & Coupland 1985), thus causing glyphosate-treated lavender to no longer be a suitable foraging resource for wild bees. However, this may not be the case in plants which have been genetically modified to be more tolerant of glyphosate, which would not experience die back following treatment. Studies which looked at the use of neonicotinoid insecticides found that bees *(Bombus terrestris audax*) preferred untreated resources when thiamethoxam was first used and applied to the environment, but over time workers developed an acquired preference for thiamethoxam treated plants where visitation and consumption of food containing thiamethoxam increased (Arce et al. 2018). This suggests that in the beginning bumble bees avoided foraging on treated resources, but with increased exposure to the insecticide, treated

crops became increasingly attractive to foraging workers (Arce et al. 2018; Kessler et al. 2015). This may well be the case in large scale agricultural crop production settings where glyphosate-tolerant crops are becoming the norm (Padgette et al. 1995; Green 2016). In fact, in 2017 a study found that foraging honey bee workers presented a preference for glyphosate-sugar water in comparison with control sugar water (Liao, Wu & Berenbaum, 2017). This is a cause for concern considering the use of glyphosate-tolerant crops is increasing (Padgette et al. 1995; Green 2016), leading in an increased use of glyphosate-based herbicides as growers are no longer concerned about crop die back. With the overuse of glyphosate, the evolution of glyphosate resistant weeds are becoming more abundant (Duke & Powles 2008a), and to combat this it is expected growers will begin using higher concentrations of glyphosate in attempt to control resistant weeds, thus increasing potential damage to the pollinator community and with it global food security (Dill 2005).

In contrast, a clear trend can be seen in visitation to control lavender over the 3 days (1hr, 24hrs and 48hrs), where visitation rates increased with time after treatment application. A reason for increased visitation from 1hr to 24hrs and from 24hrs to 48hrs could be that foraging workers may be able to detect glyphosate, and although control flowers remain appropriate resources, they may be avoided as they lie within close proximity to the treated plants (Desmedt et al. 2016; Kremer et al. 2005). This could mean that, although an abundance of resources are available to use for feeding and nesting, bees may be left with no 'suitable' resources due to their avoidance of untreated plants neighbouring those that have been treated with glyphosate-based herbicides. This is supported by the results obtained in a study by Kremer et al., that found plants treated with glyphosate can cause growth inhibition of neighbouring plants and seedlings through the process of exudation (Kremer et al. 2005), thus suggesting that non-target plants adjacent to those that are treated suffer indirectly from glyphosate application. This leads to concern that resources, once believed to be suitable foraging and nesting sites for bees, may no longer be enough to sustain bee populations causing further declines, and potentially having an impact on global food security. However, increased visitation to control lavender over the 3-day period could be a result of food recruitment (Nieh, 1998), for instance, a worker bee could have encountered and fed on the lavender on the first day, and once returning to the hive, could have performed the waggle dance (Dornhaus & Chittka 2001), communicating the position of the experimental lavender to others in their colony, thus leading to increased visitation over the three day period.

4.3 PARASITE VULNERABILITY

Although insignificant, a clear trend can be seen in the spore count in treated colonies over a 3-week period (Table 4; Figure 12). The results obtained suggest that those fed with glyphosate laced sugar solution were more vulnerable to pathogen infestation than those in control hives. A possible explanation for this could be that exposure to glyphosate interacts with bee gut microbiota (Motta, Raymann & Moran 2018). Bees, much like humans, rely on numerous enzymes and a community of bacteria for the proper functioning of their digestive system, regulation of their immune system and for protection against pathogens (Motta, Raymann & Moran 2018). Previous studies have found that glyphosate, due to its role in the inhibition of 5-enolpyruvylshikimate-3-phosphatesynthase (EPSPS), increases the likelihood of honey bees contracting pathogens (Raymann, Shaffer & Moran 2017; Raymann, Bobay & Moran 2018; Schwarz, Moran & Evans 2016). In addition, another study in 2018 found that a community of microbiota is essential to protect honey bees from opportunistic pathogens (Motta, Raymann & Moran 2018). This suggests that the increased use of glyphosate within agricultural crop production has the potential to cause further declines in bee population numbers, leading to concern over global food production and the pollination services of many other plant species.

The spore count in control colonies on day one came as a surprise, as it was expected that treated colonies would be more susceptible to pathogen infestations than control. However, because the spore count on day 1 is higher than that found at week 1 or week 3 it is assumed that the count obtained on day 1 is an anomaly or a product of human error. Although both treatment groups became increasingly infected over the 3-week period, treated colonies presented a higher spore count than controls in week 1 and week 3. Therefore, if results were observed in isolation of control day 1, there is an obvious trend, although insignificant, that control colonies are less vulnerable to pathogen infestation than those treated with glyphosate laced sugar solution. Studies conducted in the past have set out to investigate the relationship between pesticide exposure on bees' pathogen infestation rate (Collison et al. 2015). One study by Pettis et al., found that honey bees exposed to pesticides were found to be significantly more infected than untreated hives. (Pettis et al. 2012). This is a cause for concern as both pesticide exposure and pathogens are considered leading causes of pollinator losses, and the interaction between the two can lead to further declines in bee populations.

However, it is important to note that the method, although standard procedure (Fries et al. 2013) for measuring the level of colony infection by *Nosema* spores has been found to be

unreliable. Studies conducted in the past found that spore count do not share a direct relationship to the parasite burden and health of a whole colony (Higes et al. 2008)

4.4 Colony Productivity

Number of workers

Glyphosate may impact bees' cognitive function and capabilities, specifically bees exposed to glyphosate could experience impaired navigation or spatial processing, compromising an individual's ability in returning to the hive (Balbuena et al. 2015; Herbert et al. 2014). This could explain why the number of workers is lower in colonies which were treated with glyphosate laced sugar solution as opposed to control hives. In fact, the results obtained in a study by Balbuena et al support our findings, where it was found that honey bees' spatial learning was impaired following the ingestion of glyphosate. Although this study was carried out in honey bees, similar effects on *Bombus spp.* could be expected. In extension, honey bees' spatial learning ability deteriorated as the concentration of glyphosate was increased (Balbuena et al. 2015), which is an interesting and extremely relevant result, especially in today's society with the evolution of glyphosate-resistant weeds and the increasing likelihood that growers will begin to use higher concentrations of glyphosate in an attempt to control herbicide resistant weeds (Dill 2005). This leads to concern that the already declining bee population will continue to experience losses due to the increased probability that workers will be unable to return to the hive after foraging.

Reproductive Output

When considering the population genetics of both glyphosate-treated and control colonies, it is suggested that exposure to glyphosate had an effect on colony reproductive output. Results suggest that treated colonies experienced a reduction in worker numbers yet presented higher numbers of brood cells. It is unclear if eclosion in treated colonies was delayed in due to direct impacts of treatment on larvae or indirect impacts of exposure to glyphosate i.e. poor provisioning (Feltham, Park & Goulson 2014; Gill Ramos-Rodriguez & Raine 2012). Moreover, the reduction of workers in treated colonies led to alterations in life-history trajectories of both control and glyphosate-treated colonies, with effects on reproductive output in treated colonies being a consequence of glyphosate exposure (Bryden et al. 2013). Results obtained are somewhat similar to those found in studies that focused on

the effects of neonicotinoids where those treated with neonicotinoids presented a 32-36% decrease in the mean production of workers and/or drones (Woodcock et al. 2017).

Sex Ratio

Our results show that treated colonies produced less drones than controls. Although this study used a limited samples size; 3 control and 3 treated colonies (n=6) it should be considered that exposure to glyphosate potentially had an impact on the number of drones produced, where treated colonies produced 100% less drones than controls. Our results fall in line with a previous study which looked at the impact of sulfoxaflor on reproductive success, where it was found that although both treatment groups were equally as likely to produce males, treated colonies in total produced significantly fewer males (Siviter, Brown & Leadbeater 2018). Similar results have been found in colonies exposed to neonicotinoids (Woodcock et al. 2017). If exposure to glyphosate works in a similar way to that of sulfoxaflor and neonicotinoids, then it could be assumed, given a larger sample size, the number of drones produced may be significantly different across treatment groups, suggesting that exposure to glyphosate could potentially cause environmental impacts similar to those of neonicotinoids (Siviter, Brown & Leadbeater 2018).

Colony Weight

Results show that treated colonies were 500% heavier than control colonies (Table 5), which could suggest that treated colonies prioritised colony growth over reproduction, although treated colonies were seen to have 240% more brood cells than those seen in controls. This could further explain that treated colonies delayed the reproduction phase of their colony cycles until later in the 3-week period than control colonies. Such results could again be caused by behaviour changes where exposure to glyphosate caused hyperactivity (Boily et al. 2013), thus causing bees to go out and forage at a higher rate than those in control colonies. However, contrasting results in a study by Whitehorn et al, found that colonies exposed to neonicotinoids gained less weight than untreated controls (Whitehorn et al. 2012). However, the effects of glyphosate could differ greatly to the effects of neonicotinoid pesticides on bee productivity.

Supplementary feeder consumption

Results show that colonies fed with glyphosate laced sugar solution consumed 132% more sugar solution than those in control colonies. This suggests the presence of glyphosate influenced bees within treated hives to consume larger volume of sugar solution. Past studies have found that over time bees will show preference of pesticide treated plants, found in both neonicotinoid (Arce et al. 2018; Kessler et al. 2015) and glyphosate studies (Liao, Wu & Berenbaum 2017). This suggests that acute exposure to field realistic concentrations of glyphosate (up to 3.7 mg l^{-1} GLY; Giesy et al. 2000) over time can lead to an acquired preference of treated foraging resources. In this case, pollinator-dependent crops would unlikely experience reductions in pollination. However, a preference for treated plants could have huge potential effects on wildflower species. Potentially leading to the degradation of crucial natural food webs, and inadvertently affecting crop production due to further pollinator losses.

4.5 LIMITATIONS OF STUDY

However, some limitations should be noted;

- **Sample size:** Although external funding was achieved the majority of the money was spent on experiment one colonies. The weather conditions during experiment one meant that all experiment one colonies were no longer useable. This meant that there was limited funds to spend on replacement colonies which greatly reduced the sample size. The sample size used during this research was less than desirable, thus making it difficult to find significant relationships from the data, as large sample sizes are required to perform statistical tests that consider a representative distribution.

- **Lack of prior research:** Studies similar to this have looked at the effects of pesticides other than glyphosate, i.e. neonicotinoids, on bee health and foraging ability, which were often performed on honey bees. Therefore, it could be argued that some of the studies used to support our findings are not reliable as they present the effects of different chemicals on different species of bees.

- **Weather conditions:** The weather conditions during both experiment one and two may have influenced both productivity and health in the commercial colonies. This is because

when unfavourable weather conditions occur, bees are unable to exit to forage or perform cleansing flights, decreasing time spent foraging, thus lowering productivity. This also increases the likelihood of bees defecating within the hive, potentially facilitating the spread of disease within the colony.

5. Conclusion and Future Research

In conclusion, the results obtained suggest that exposure to glyphosate can indeed have an impact on bumble bee foraging ability and behaviour, productivity and pathogen vulnerability. Although insignificant, which is likely a result of the small sample size, clear trends can be seen. The study therefore could provide an overview of the possible effects of field-realistic cumulative concentrations of glyphosate on wild bee populations and could be used as a basis for future research.

It is becoming increasingly important that future research looks at both individual level behavioural effects of pesticide exposure and its impacts on wild bee populations. The majority of studies conducted in the past have focused on the more commercialised honey bee – *Apis melifera*. Although results from such studies can be used as the basis for the potential impacts of pesticide exposure on wild bee populations, it is important that future research sets out to investigate the specific effects of multiple pesticides on wild bees such as *Bombus spp.*

This study used field-realistic concentrations of glyphosate (up to 3.7 mg l^{-1} Glyphosate; Giesy et al. 2000) which bees may encounter in the natural environment, however with the increased rate of herbicide-resistant weeds, farmers are choosing to use higher concentrations than what is recommended for glyphosate use. Thus, the concentration of glyphosate which foraging bees may encounter will increase. It is therefore important that future studies set out to investigate the impacts of sub-lethal concentrations of Glyphosate on the function and health of unmanaged bumble bees.

Additionally, the potential interactions between different pesticides could be investigated to look at combined effects on bee health, function and behaviour, as it is unrealistic that bees, when out foraging, would encounter only one type of pesticide in isolation of the others.

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7. APPENDIX

Grid References (obtained from google maps):

Wildlife Garden: 54°34'59.8"N 5°55'54.4"W

Lennoxvale Lake: 54°34'35.3"N 5°56'13.4"W

Lock Keeper's Inn: 54°33'13.2"N 5°56'41.2"W

Table 7.1 Complete weather data set during experiment 1 (6th June – 8th July), consisting of max and min temperature and total rainfall in mm

Experiment one Weather Data 2009-2019

YYYY	mm	dd	Max Temp °C	Min Temp °C	Rainfall (mm)
2009	6	6	11	6	0.76
2009	6	7	14	8	0
2009	6	8	15	9	0.04
2009	6	9	15	9	0
2009	6	10	15	7	0.18
2009	6	11	14	7	0
2009	6	12	14	1	0
2009	6	13	17	11	3.07
2009	6	14	17	9	4.64
2009	6	15	17	8	2.05
2009	6	16	18	5	0.7
2009	6	17	14	11	9.61
2009	6	18	14	10	1.85
2009	6	19	14	10	0.88
2009	6	20	14	11	0.55
2009	6	21	15	10	2.44
2009	6	22	20	14	1.28
2009	6	23	22	13	0.14
2009	6	24	19	10	0.01
2009	6	25	19	13	0
2009	6	26	21	13	0.24
2009	6	27	21	13	0.34
2009	6	28	21	13	0.07
2009	6	29	23	14	1.16
2009	6	30	22	14	0
2009	7	1	22	15	0.3
2009	7	2	19	16	3.01
2009	7	3	20	15	5.01
2009	7	4	17	13	2.2
2009	7	5	18	12	0.85
2009	7	6	18	12	4.69
2009	7	7	17	13	0.19
2009	7	8	16	12	0.08
2010	6	6	15	10	0.92
2010	6	7	17	7	0.49
2010	6	8	12	11	8.32
2010	6	9	14	11	1.55
2010	6	10	17	10	0.2
2010	6	11	15	7	0.11
2010	6	12	16	10	0.12
2010	6	13	15	9	3.71
2010	6	14	16	10	0
2010	6	15	19	8	0
2010	6	16	21	8	0.03
2010	6	17	19	11	0
2010	6	18	19	11	0.16
2010	6	19	15	10	0
2010	6	20	18	9	0
2010	6	21	21	10	0.02
2010	6	22	19	14	0.01
2010	6	23	18	15	1.24

2010	6	24	17	11	0.06
2010	6	25	19	14	0.01
2010	6	26	19	13	1
2010	6	27	19	13	1.48
2010	6	28	17	12	3.9
2010	6	29	19	12	0
2010	6	30	20	13	1.37
2010	7	1	20	14	4.92
2010	7	2	19	13	0.9
2010	7	3	16	12	0.57
2010	7	4	17	12	1.86
2010	7	5	15	12	0.16
2010	7	6	16	9	4.56
2010	7	7	17	13	0.3
2010	7	8	17	12	0.37
2011	6	6	12	7	0.24
2011	6	7	12	9	12.31
2011	6	8	12	8	5.57
2011	6	9	10	5	3.49
2011	6	10	12	5	0.21
2011	6	11	13	7	1.14
2011	6	12	12	5	0
2011	6	13	15	10	0.33
2011	6	14	14	7	0
2011	6	15	16	11	1.48
2011	6	16	14	8	1.4
2011	6	17	13	8	7.94
2011	6	18	14	8	1.13
2011	6	19	14	9	1.26
2011	6	20	15	7	0.68
2011	6	21	15	11	7.5
2011	6	22	14	10	1.46
2011	6	23	13	9	0.21
2011	6	24	13	7	3.17
2011	6	25	18	11	0.77
2011	6	26	18	12	1.47
2011	6	27	17	12	2.37
2011	6	28	16	7	0.94
2011	6	29	14	8	1.62
2011	6	30	14	8	0
2011	7	1	16	6	0
2011	7	2	16	8	0
2011	7	3	18	10	0.14
2011	7	4	17	12	0.09
2011	7	5	16	13	4.54
2011	7	6	15	10	1.19
2011	7	7	15	7	1.8
2011	7	8	15	12	0.6
2012	6	6	15	7	1.65
2012	6	7	13	10	6.73
2012	6	8	13	11	1.26
2012	6	9	13	10	0.18
2012	6	10	15	5	0
2012	6	11	14	5	0
2012	6	12	13	9	0.51
2012	6	13	14	9	1.88
2012	6	14	13	9	2.8
2012	6	15	12	9	3.12
2012	6	16	14	10	6.12
2012	6	17	13	8	0.28
2012	6	18	13	3	1.98
2012	6	19	14	8	1.47
2012	6	20	15	8	0.55
2012	6	21	12	11	2.11
2012	6	22	11	10	15.38
2012	6	23	14	9	3.79
2012	6	24	15	10	0
2012	6	25	15	10	0.05
2012	6	26	17	9	1.09
2012	6	27	19	14	7.89
2012	6	28	16	13	3.36
2012	6	29	16	12	1.16

2012	6	30	14	11	0.37
2012	7	1	14	10	2.93
2012	7	2	16	12	0.34
2012	7	3	15	13	1.09
2012	7	4	17	12	0.35
2012	7	5	17	8	4.87
2012	7	6	17	13	0
2012	7	7	17	14	4.27
2012	7	8	13	12	2.55
2013	6	6	16	6	0
2013	6	7	18	9	0
2013	6	8	17	9	0.01
2013	6	9	17	8	0
2013	6	10	16	10	0
2013	6	11	14	10	3.42
2013	6	12	15	10	3.17
2013	6	13	14	10	2.67
2013	6	14	15	8	5.13
2013	6	15	13	7	1.65
2013	6	16	15	8	0.34
2013	6	17	16	9	0.57
2013	6	18	18	8	0.25
2013	6	19	16	9	0.12
2013	6	20	15	9	0.01
2013	6	21	15	12	2.12
2013	6	22	14	11	0.33
2013	6	23	12	10	0.42
2013	6	24	13	8	0.6
2013	6	25	16	7	1
2013	6	26	15	9	0
2013	6	27	15	10	6.1
2013	6	28	15	12	0.45
2013	6	29	16	9	0.31
2013	6	30	14	11	0.52
2013	7	1	14	9	0.51
2013	7	2	14	10	1.82
2013	7	3	16	11	0
2013	7	4	16	12	0.18
2013	7	5	17	9	0
2013	7	6	19	12	0.03
2013	7	7	20	15	0.02
2013	7	8	21	12	0
2014	6	6	15	6	0.01
2014	6	7	15	12	5.44
2014	6	8	16	10	0.57
2014	6	9	15	11	0.25
2014	6	10	16	11	2.06
2014	6	11	17	10	0.76
2014	6	12	18	11	0.06
2014	6	13	18	13	0.13
2014	6	14	18	12	0
2014	6	15	17	10	0
2014	6	16	18	11	0
2014	6	17	20	11	0.04
2014	6	18	19	12	0
2014	6	19	16	11	0.1
2014	6	20	15	10	0.1
2014	6	21	16	10	0.18
2014	6	22	17	10	0.02
2014	6	23	17	10	0.86
2014	6	24	16	11	0
2014	6	25	14	10	1.05
2014	6	26	14	12	1.75
2014	6	27	15	12	2.81
2014	6	28	14	10	0.19
2014	6	29	17	9	0.18
2014	6	30	17	6	0
2014	7	1	19	8	0
2014	7	2	18	13	0.27
2014	7	3	17	11	0.48
2014	7	4	16	11	3.81
2014	7	5	16	6	0.87

2014	7	6	16	9	2.55
2014	7	7	16	10	1.56
2014	7	8	15	11	1.58
2015	6	6	13	8	0.75
2015	6	7	13	6	0.36
2015	6	8	13	4	0.12
2015	6	9	15	4	0
2015	6	10	16	5	0
2015	6	11	16	7	0
2015	6	12	17	8	0
2015	6	13	14	10	0
2015	6	14	14	7	0.06
2015	6	15	14	3	0.11
2015	6	16	18	12	0.16
2015	6	17	16	12	9.69
2015	6	18	16	12	0.06
2015	6	19	15	10	1.69
2015	6	20	16	12	0.01
2015	6	21	14	9	1.73
2015	6	22	16	8	0.49
2015	6	23	18	7	0
2015	6	24	17	8	2.59
2015	6	25	16	11	2.35
2015	6	26	19	12	3.03
2015	6	27	16	9	0.27
2015	6	28	17	13	8.49
2015	6	29	18	10	0.52
2015	6	30	22	14	0.07
2015	7	1	22	14	1.18
2015	7	2	19	13	1.28
2015	7	3	19	11	0.01
2015	7	4	19	13	1.24
2015	7	5	17	10	0.91
2015	7	6	16	9	3.62
2015	7	7	16	13	1.7
2015	7	8	15	10	0.03
2016	6	6	22	11	0.34
2016	6	7	19	12	2.15
2016	6	8	20	13	0
2016	6	9	20	10	0.04
2016	6	10	15	13	0.08
2016	6	11	18	12	0.74
2016	6	12	16	10	0.73
2016	6	13	16	10	0.77
2016	6	14	15	9	0.73
2016	6	15	12	10	0
2016	6	16	16	9	1.33
2016	6	17	16	11	0.25
2016	6	18	15	8	0.05
2016	6	19	16	11	7.69
2016	6	20	16	12	0.24
2016	6	21	16	12	1.58
2016	6	22	16	11	0.88
2016	6	23	18	9	2.21
2016	6	24	15	9	3.59
2016	6	25	16	10	0
2016	6	26	15	8	0.67
2016	6	27	15	10	0.29
2016	6	28	13	9	2.85
2016	6	29	14	9	4.71
2016	6	30	14	8	1.43
2016	7	1	13	9	0.5
2016	7	2	14	8	0.29
2016	7	3	15	8	0.11
2016	7	4	14	8	3.45
2016	7	5	14	9	0.21
2016	7	6	15	6	3.37
2016	7	7	17	12	1.02
2016	7	8	17	13	1
2017	6	6	14	9	0.04
2017	6	7	14	10	0.2
2017	6	8	15	11	3.24

2017	6	9	17	9	0.11
2017	6	10	17	11	5.68
2017	6	11	15	12	1.86
2017	6	12	15	12	0.25
2017	6	13	17	12	3.2
2017	6	14	16	12	0.01
2017	6	15	15	12	1.52
2017	6	16	18	12	0.27
2017	6	17	20	13	0.01
2017	6	18	21	13	0.1
2017	6	19	19	12	0.6
2017	6	20	19	9	0
2017	6	21	22	15	2.29
2017	6	22	15	13	0.24
2017	6	23	16	13	4.16
2017	6	24	14	10	0
2017	6	25	14	10	0.04
2017	6	26	15	7	1.07
2017	6	27	17	10	3.07
2017	6	28	14	12	0.1
2017	6	29	12	11	4.43
2017	6	30	14	11	1.03
2017	7	1	15	7	0.02
2017	7	2	15	9	0.2
2017	7	3	15	11	1.93
2017	7	4	15	11	11.48
2017	7	5	16	9	0.1
2017	7	6	18	12	1.71
2017	7	7	16	11	1.84
2017	7	8	18	7	0
2018	6	6	18	11	0
2018	6	7	20	12	0.37
2018	6	8	18	12	1.43
2018	6	9	19	11	3.47
2018	6	10	17	11	0.04
2018	6	11	16	10	0.12
2018	6	12	14	10	0
2018	6	13	14	9	3.88
2018	6	14	15	11	0.67
2018	6	15	13	9	2.27
2018	6	16	14	9	4.23
2018	6	17	16	10	0.42
2018	6	18	15	9	0.21
2018	6	19	16	7	2.65
2018	6	20	16	10	1.59
2018	6	21	13	8	0.01
2018	6	22	15	7	0
2018	6	23	17	7	0
2018	6	24	19	9	0
2018	6	25	21	13	0
2018	6	26	20	13	0
2018	6	27	22	14	0
2018	6	28	24	15	0
2018	6	29	22	15	0
2018	6	30	20	15	0
2018	7	1	19	13	0
2018	7	2	20	11	0
2018	7	3	19	13	0
2018	7	4	21	13	0.02
2018	7	5	17	12	0
2018	7	6	18	10	0
2018	7	7	21	13	0.16
2018	7	8	20	15	0.65
2019	6	6	14	8	2.9
2019	6	7	14	7	1.8
2019	6	8	13	9	25.3
2019	6	9	13	6	11.3
2019	6	10	15	8	1.1
2019	6	11	14	9	0.6
2019	6	12	12	9	1.9
2019	6	13	13	8	2.3
2019	6	14	13	7	5.2

2019	6	15	13	8	13.6
2019	6	16	14	6	5.2
2019	6	17	15	7	2.5
2019	6	18	16	7	2.5
2019	6	19	15	8	1.9
2019	6	20	14	7	2.1
2019	6	21	15	4	1.7
2019	6	22	16	8	0.1
2019	6	23	16	9	2.7
2019	6	24	17	11	9.5
2019	6	25	18	10	0.1
2019	6	26	18	10	0
2019	6	27	19	12	0
2019	6	28	19	13	0.3
2019	6	29	21	14	1.7
2019	6	30	17	11	3.6
2019	7	1	16	11	1
2019	7	2	15	7	0.7
2019	7	3	17	6	0.1
2019	7	4	17	9	0.2
2019	7	5	18	9	1.1
2019	7	6	16	10	0.8
2019	7	7	17	7	0.1
2019	7	8	14	8	5.1

a)

b)

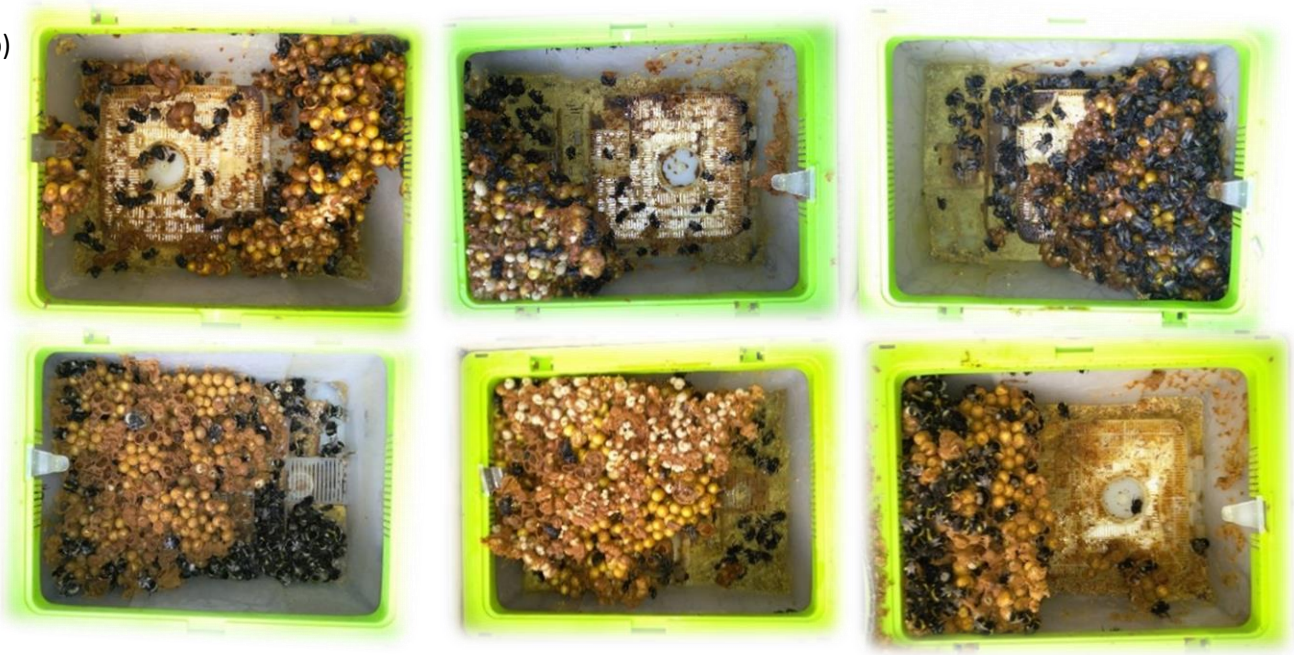

Figure 7.1 Photographs of colonies used during experiment one

a) Notable signs of dysentery seen on the front of hives
b) Photograph of the inside of colonies which presented signs of dysentery

Table 7.2 Complete weather data set during experiment 1 (26th July – 16th August), consisting of max and min temperature and total rainfall in mm

Experiment Two Weather Data

YYYY	mm	dd	Max Temp °C	Min Temp °C	Rainfall (mm)
2019	7	26	19	14	2
2019	7	27	16	12	6.1
2019	7	28	14	9	15.1
2019	7	29	19	12	11.8
2019	7	30	18	12	0.5
2019	7	31	18	12	0.6
2019	8	1	19	10	0
2019	8	2	19	12	0
2019	8	3	19	13	4.5
2019	8	4	19	14	10.9
2019	8	5	18	12	8.6
2019	8	6	17	12	12
2019	8	7	18	10	9.2
2019	8	8	19	10	0.4
2019	8	9	19	14	27
2019	8	10	18	13	22.6
2019	8	11	14	9	4.6
2019	8	12	15	7	3
2019	8	13	16	6	3
2019	8	14	16	11	6.6
2019	8	15	17	13	0.9
2019	8	16	19	12	9.1

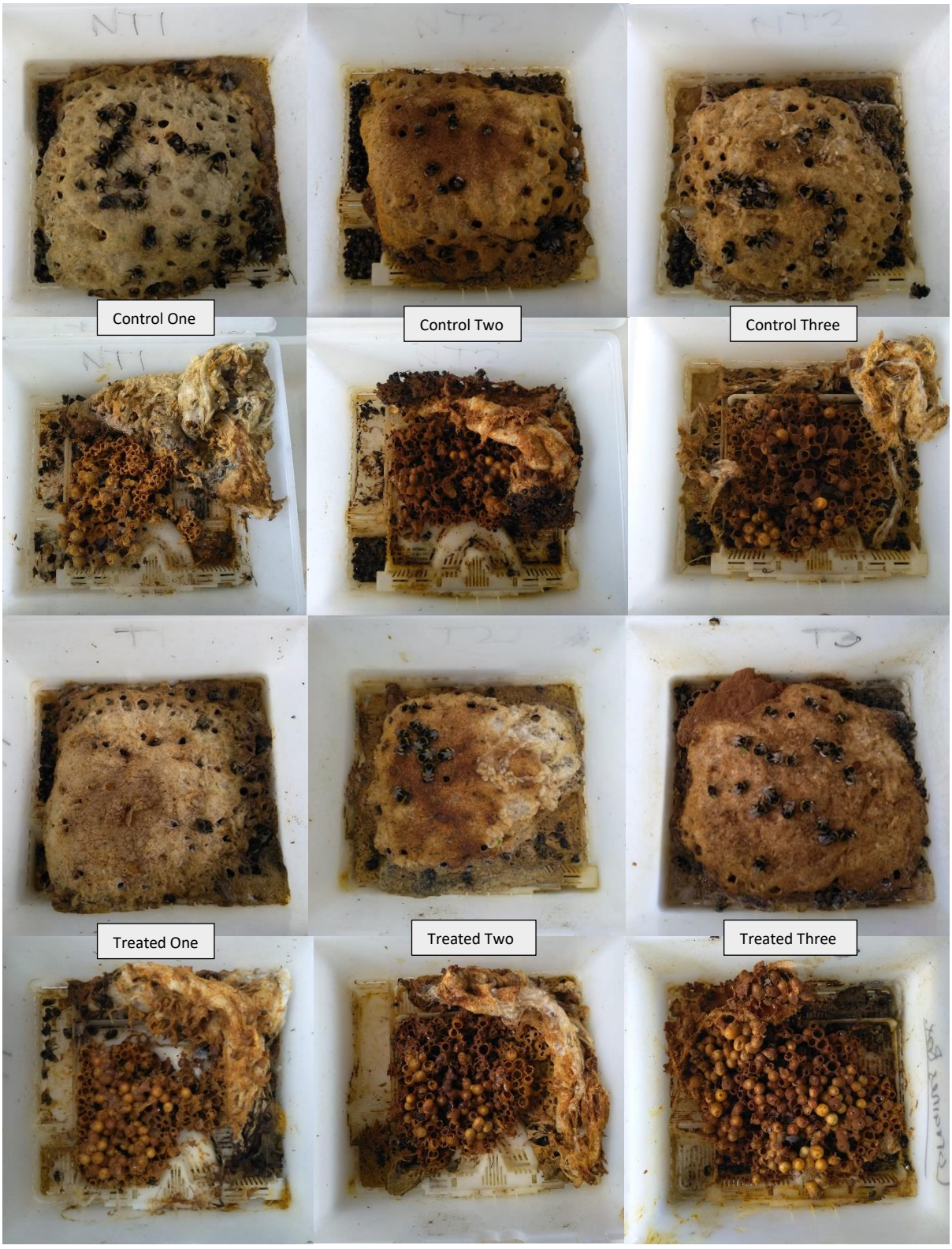

Figure 7.2 Photographs of the nest inside each of the colony boxes; controls (top two rows), treated (bottom two rows)

Table 7.3 Complete weather data set during wild observations consisting of temperature and total rainfall in mm

Wild Observation Weather Data

YYYY	mm	dd	Temp °C	Rainfall (mm)
2019	7	15	19	0.1
2019	7	16	19	0.8
2019	7	17	15	3.6
2019	7	22	20	0.1
2019	7	23	21	0
2019	7	24	21	0.1
2019	7	26	19	0
2019	7	27	16	0
2019	7	28	13	7.1
2019	7	29	18	0.2
2019	7	30	16	0
2019	7	31	16	0.2
2019	8	5	16	0
2019	8	6	17	1.6
2019	8	7	17	0.6

Table 7.4 Record of both external parasites and presence of *Nosema* spores within samples over taken over a 3-week period. (C# = control colony number, T# = treated colony number D1 = day one, W1 = Week one W3 = Week three)

	P. fucorum	Rep 1	Rep 2	TOTAL Number of spores counted	*Average no. of N. ceranae spores per square*	Spores/ml	Spores/bee	
C1W1	No	1	2	3	1.5	1.5	300000	3.0E+05
C2W1	No	2	1	3	1.5	1.5	300000	3.0E+05
T1D1	No	1	2	3	1.5	1.5	300000	3.0E+05
T1W1	No	2	2	4	2	2	400000	4.0E+05
T2D1	No	3	1	4	2	2	400000	4.0E+05
T2W3	no	2	2	4	2	2	400000	4.0E+05
T3W1	No	2	3	5	2.5	2.5	500000	5.0E+05
Old 2	No	4	1	5	2.5	2.5	500000	5.0E+05
C1D1	No	2	4	6	3	3	600000	6.0E+05
C2W3	no	2	4	6	3	3	600000	6.0E+05
T3D1	No	4	2	6	3	3	600000	6.0E+05
Old 3	No	2	4	6	3	3	600000	6.0E+05
C3W1	No	5	2	7	3.5	3.5	700000	7.0E+05
C3W3	no	2	5	7	3.5	3.5	700000	7.0E+05
C2D1	No	5	3	8	4	4	800000	8.0E+05
T2W1	No	2	6	8	4	4	800000	8.0E+05
C1W3	no	6	5	11	5.5	5.5	1100000	1.1E+06
C3D1	No	5	6	11	5.5	5.5	1100000	1.1E+06
T3W3	yes	5	6	11	5.5	5.5	1100000	1.1E+06
Old 1	No	7	7	14	7	7	1400000	1.4E+06
T1W3	Yes	7	9	16	8	8	1600000	1.6E+06