

**Bangor University**

## **DOCTOR OF PHILOSOPHY**

### **The sublethal effects of chronic agrochemical exposure in the buff-tailed bumblebee (*Bombus terrestris audax*)**

Oliver, Thomas

*Award date:*  
2023

*Awarding institution:*  
Bangor University

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**The sublethal effects of chronic agrochemical  
exposure in the buff-tailed bumblebee  
(*Bombus terrestris audax*)**

Thesis submitted for the degree of Doctor of Philosophy (PhD)

University of Bangor

March 2023

*Thomas Richard Oliver*



PRIFYSGOL  
**BANGOR**  
UNIVERSITY



**ROTHAMSTED  
RESEARCH**

## Author declaration

I hereby declare that this thesis is the results of my own investigations, except where otherwise stated. All other sources are acknowledged by bibliographic references. This work has not previously been accepted in substance for any degree and is not being concurrently submitted in candidature for any degree unless, as agreed by the University, for approved dual awards.

I confirm that I am submitting this work with the agreement of my Supervisors.

---

Yr wyf drwy hyn yn datgan mai canlyniad fy ymchwil fy hun yw'r thesis hwn, ac eithrio lle nodir yn wahanol. Caiff ffynonellau eraill eu cydnabod gan droednodiadau yn rhoi cyfeiriadau eglur. Nid yw sylwedd y gwaith hwn wedi cael ei dderbyn o'r blaen ar gyfer unrhyw radd, ac nid yw'n cael ei gyflwyno ar yr un pryd mewn ymgeisiaeth am unrhyw radd oni bai ei fod, fel y cytunwyd gan y Brifysgol, am gymwysterau deuol cymeradwy. Rwy'n cadarnhau fy mod yn cyflwyno'r gwaith hwn gyda chytundeb fy Ngoruchwyliwr (Goruchwylwyr).

Thomas Richard Oliver

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To my friends, for giving me a sense of belonging during my time at Rothamsted. Even after the pandemic, you still made the effort to ensure everyone was included in social gatherings online. Thank you for helping me to feel connected and to feel a little less lonely. To Donna Fellows for always being so positive, helpful, and just a great person all round. A huge thank you to Mark Wilkinson, who stepped up when no one else did to ensure that I was ok and well supported when things were going wrong. You can stop apologising now.

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## External impacts

A number of external factors outside of my control have shaped this thesis. The original title of my project was “Dispersal strategies and space use in pollinating bees using a novel drone tracking technology”. My project was funded on the basis of a new form of tracking technology developed at Bangor University. This technology consisted of a new type of telemetric tag and a drone capable of tracking said tag. During my first field season in 2019, the drone was not yet finished but I was told the tags and tracking array were ready for use. I attempted to conduct two field studies during the summer of 2019 at Rothamsted Research using the tag and tracking array but was plagued by technical issues that could not be resolved by the engineers at Bangor University.

During spring 2020, I attempted to conduct fieldwork, again, using the technology on which my PhD was secured. The technology was not functional, and during attempts to fix it, COVID-19 restrictions began. After a year of unsuccessful attempts to use this technology to collect my data, it was agreed during my second year review in June 2020 that I would begin work on a backup plan to make up for the time I had lost from the previous summer and COVID-19 once restrictions were lifted. The backup plan involved the use of the harmonic radar system at Rothamsted Research. I began conducting my research, with the help of Dr Joe Woodgate, in early July 2020. However, due to an accident that took place at Rothamsted Research in late 2019 and following on from a subsequent Health & Safety England inspection, it transpired that we were no longer allowed to conduct our research. The buildings containing the radar systems were made inaccessible to my colleague and me only three days after we started our research, and they remained closed to us until November 2020. During this time, the PhD student working on the new drone tracking technology at Bangor University submitted their thesis but unfortunately for me, did not complete the drone.

Thus, Chapter 1 is a systematic map covering the history of insect telemetry and the progress of tag-based telemetry over the last 9 years. It is the first such review since Kissling *et al.* published the only review of insect telemetry in 2014. Whereas, Chapters 2, 3 & 4 pertain to agrochemical exposure on *Bombus terrestris audax* flight characteristics as recorded in a flight

arena using visual observations and on flight mills in controlled environments. This is followed by a general discussion.

## Summary

The rationale for this PhD research project focuses on addressing a critical knowledge gap concerning the potential consequences of chronic sublethal exposure to agrochemicals on bumblebee foraging activity and flight characteristics. Agrochemicals, commonly used in modern agriculture, have been a subject of concern due to their potential impact on pollinator populations, including essential bumblebee species.

Despite the extensive use of agrochemicals and their potential association with pollinator decline, there remains a lack of comprehensive understanding regarding the sublethal effects of these chemicals on bumblebees' behaviour and flight patterns. Previous research has predominantly focused on acute toxicity, overlooking the more subtle, yet equally significant, long-term impacts that chronic sublethal exposure might have on bumblebee colonies and their ecological functions.

Thus, the aim of this thesis was to quantify the potential impact of chronic sublethal exposure of agrochemicals on buff-tailed bumblebee (*Bombus terrestris audax*) foraging activity and flight characteristics. The first chapter presents a systematic review of the current state of the field of insect telemetry, highlighting the need for future work to focus on quantifying the impact of tracking techniques on a broader range of insect taxa. The second and third chapters investigate the impact of sulfoxaflor and flupyradifurone, alone and in combination, on bumblebee flight characteristics and foraging activity. The fourth chapter investigates the impact of flupyradifurone and boscalid on bumblebee flight characteristics.

The second chapter reports bumblebee workers exposed to sulfoxaflor had reduced average and maximum flight speed and increased distance flown per flight bout. The third chapter reports exposure to flupyradifurone, sulfoxaflor, and both together increased the average and maximum flight speed of bumblebees, while the propensity of individuals to initiate flight was significantly higher in the groups treated with sulfoxaflor and sulfoxaflor with flupyradifurone. However, there were no significant differences in the distributions of flight and pause durations. The fourth chapter reports exposure to flupyradifurone and boscalid, alone and in

combination, significantly increased flight speed, flight distance, and flight duration, while the pause duration between successive flights was significantly longer in the boscalid group.

Overall, the findings indicate that agrochemicals have sublethal impacts on bumblebee flight and foraging ability, and can interact with one another. The thesis highlights the need for a mechanism of interaction between these chemicals to be identified, and for future research to broaden the focus beyond the Apidae family and to quantify the impact of tracking techniques on a broader range of insect taxa.

Following the ban of highly controversial neonicotinoid pesticides' there exists a desire for alternative agrochemicals. Two of these alternatives, flupyradifurone and sulfoxaflor, share a number of molecular and chemical characteristics with neonicotinoids. They are an alternative to neonicotinoids and are in high demand in areas with high neonicotinoid resistance. Ergo, non-target organisms, such as the bumblebee, will likely be exposed to these novel agrochemicals in agricultural environments. The effect these chemicals may have on beneficial organisms necessitates quantification to ensure we do not have a repeat of the case of neonicotinoid pesticides.



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## Prologue

### Pollinators

Pollinators play a crucial role in the functioning of various terrestrial ecosystems, particularly in agricultural-dominated ones, where the interaction between plants and pollinators significantly influences plant productivity (1). The extent to which the world's 352,000 angiosperm species rely on animal pollination has been subject to different estimates. Early approximations ranged from 67% to 96% (2, 3). However, more recent studies suggest that approximately 80% to 87.5% of angiosperms depend on animal pollination in some capacity (4, 5).

Among pollinators, insects play a critical role, supporting global food production, ecosystem health, and wild plant reproduction (6-8). Approximately 35% of all food crops rely on insect pollination (6), and in Europe, about 84% of crops depend on this process (3). Bees, in particular, are considered the most vital insect pollinators (3, 9), being responsible for pollinating 90% of the world's top 107 crop types (6). Without their pollination services, it is estimated that 5-8% of the global crop supply could be lost (10).

Although the importance of bees for global crop pollination is undeniable, its assessment can sometimes be ambiguous (11), and data sources may not always be clear (6). Notably, major staple crops such as wheat, rice, and maize do not require insect pollination. Nevertheless, insect pollination remains crucial for 84% of European crop species (3), and 75% of the world's major food crops benefit from increased seed or fruit set through insect pollination (6).



The economic significance of pollination as an ecosystem service to global agriculture is substantial, with estimates ranging from \$153 billion to \$577 billion USD worldwide (9, 12, 13). This value accounts for approximately 9.5% of the world's agricultural production value per year (9).

### Insect decline

Across the globe, insect populations have been declining (14-19). These declines pose a significant threat to the stability of both natural and agricultural ecosystems, as many invertebrate species play significant roles in providing essential ecosystem services, such as pollination (4, 6, 20).

Among the invertebrates facing challenges, bees are of particular concern due to their essential role as agricultural pollinators (6, 20-23). Bumblebees (*Bombus spp.*), for example, play a pivotal role in pollinating numerous wild plants and providing valuable auxiliary crop pollination services, with an estimated value as high as \$963 per hectare (24).

Multiple factors have been linked to the decline of bumblebee populations, including intensified land use leading to resource loss (25, 26), the prevalence of parasites and diseases (27-29), and the use of pesticides (30-33). Understanding the interactions and implications of these factors is crucial for developing effective conservation strategies to protect these valuable pollinators and the ecosystems they support.

### Honeybees and bumblebees

In comparison to bumblebees, there is a wealth of data on the impact of environmental stressors on the health and performance of honeybees, including the effects of pesticides (34-37), pathogens (38), parasites (39), and inadequate nutrition (40). Perhaps this is unsurprising given that the honeybee is the only domesticated bee species and the most frequent single species of pollinator for crops worldwide (20, 41). Historically, agrochemical toxicity testing was conducted on honeybees only (42-44). Honeybees were used as proxies for all bee species when assessing the potential impact of pesticides on non-target beneficial pollinators (43). However, applying these findings directly to bumblebees must be done with caution due to the significant differences that exist between the two species, complicating the extrapolation of honeybee-specific data (45, 46).

The lifecycle of these two closely related species is very different. For example, the cycle of a honeybee colony begins in spring and involves a large increase in laying by the queen; more than 450 eggs a day (47), with the colony size peaking at approximately 40,000 individuals in mid-summer (48). During the summer, colonies often swarm. They do this by producing a large number of queen cells which hatch into virgin queens (49). The virgin queens will mate and then leave the nest with an entourage of honeybees following, usually close to 50% of the size of the colony (48). This is a swarm. The honeybees follow the queen and form a ball around her, usually on a tree branch, before she flies to a new nesting site with the swarm in tow (50). This is how honeybee colonies replicate. At the end of the summer, the colony reduces in size as the queen drastically slows her egg laying and male honeybees are evicted from the hive. They have their wings removed by worker honeybees and are physically forced from the hive entrance (48). During the winter there is very little foraging done and the colony forms a tight ball structure in the centre of the hive, with individuals pumping their flight muscles to maintain a temperature of between 17°C and 36°C in the hive (51). The lifecycle then begins anew from the beginning of spring of the following year.

In comparison, the bumblebee lifecycle begins in spring with a queen emerging from hibernation as the temperature increase signals the start of a new season. She spends the first few days foraging for nectar for herself and locating a suitable nesting site to found her colony following a random dispersal pattern (52). Once she has laid her first eggs she will begin foraging for pollen and nectar to sustain the eggs until they develop into larvae and ultimately female workers. When there are enough foragers to provide food for the entire colony, the queen will remain inside the nest for the remainder of the year laying eggs and tending to her young (53). The colony will reach a maximum peak of approximately 250 individuals (53). At the end of summer, the colony will begin producing males and virgin queens. The virgin queens leave the nest to mate and subsequently hibernate, to begin the cycle again for the following season (54).

Due to their different lifecycles and physiology, these two species will be exposed to environmental stressors in different ways. For example, because bumblebee queens hibernate - and bumblebee nests are located – underground, they may be exposed to pesticides within the soil (55). Furthermore, bumblebees are able to pollinate plant species

that honeybees cannot because they are capable of performing 'sonication' also known as 'buzz pollination' (56). Flowering plants with rigid tubular structures formed by modified anthers require a form of pollination called 'buzz pollination' in order to access the pollen (57). Bumblebees can perform this by gripping on to the anthers of the flower, uncoupling their flight muscles from their wings, and vibrating their body (58). The vibrations literally shake the pollen from the anthers, which the bumblebee then collects. Due to this evolutionary adaptation, bumblebees can forage on different plants to honeybees and hence, both species may be exposed to different chemicals and combinations of chemicals, through visiting different flowering crops. Indeed, a recent study found that honeybees and bumblebees sampled from field borders exhibited varied pesticide exposure profiles and larger bees were exposed to larger quantities of pesticide residues (59).

Due to morphological and physiological differences between honeybees and bumblebees there can be a high level of variability between responses to stressors such as agrochemicals (45, 60, 61). For example, LD50 studies have shown that bumblebee workers are similar to or less sensitive than honeybee workers for many types of pesticides (62-64).

#### Sublethal pesticide exposure

During the agrochemical licensing process, LD50 studies, the chemical dose at which 50% of a population is killed, are conducted. These studies are used to determine the upper limit of dosages that should be avoided in a field setting (43). Nonetheless, agrochemicals used at dosages far below the LD50 may still cause harm to non-target organisms.

Sub-lethal effects of pesticides occur when an organism is exposed to a relatively low dosage of a chemical that does not directly lead to death of the individual but does result in some form of impact on behaviour, fitness or longevity. Much like with honeybees being used as a proxy for all bees, historically, sub-lethal affects were not taken into account during the pesticide licensing process (44). A recent meta-analysis found that the majority of chemical risk assessments focus on lethal effects and not sub-lethal impacts or combined effects of chemicals on bees (65). However, there has been a marked increase in the number of papers published over the last decade exploring sublethal impacts of agrochemicals on bees (65).

In field realistic settings, bumblebees come in to contact with multiple harmful chemicals, over the foraging season (66). Work I conducted as part of a meta-analysis on combined impacts of stressors on bees showed that the impact of pesticides on bees, particularly multiple chemical exposure, has been underestimated (33). This extends beyond the lethal dosages tested during the regulatory process and into the realms of sublethal doses often experienced by free flying/foraging pollinators in agricultural and urban environments (67). The pesticide regulatory screening process must reflect the natural environment experienced by bees as close as possible in order to accurately simulate the real world effects of using pesticides in the field.

Following the moratorium of neonicotinoid pesticides in the EU there became a demand for replacement pesticides. As I have explained, two closely related species of bee can be exposed to chemicals via differing routes and at different dosages. They can also be exposed to multiple chemicals at the same time. As new pesticides are developed, the regulatory process must be appropriately adjusted to include all concentrations, routes, and combinations of exposure to ensure all potential outcomes have been considered before novel agrochemicals are cleared for use.

As such, I feel there is a need to quantify the sublethal impacts of novel agrochemicals, alone and in combination, on bumblebees. Therefore, the objectives of this thesis are:

- To quantify the effects of the neonicotinoid replacement Sulfoxaflor on bumblebee flight behaviour
- To quantify the effects of neonicotinoid replacements, Sulfoxaflor and Flupyradifurone, on bumblebee flight behaviour alone and in combination
- To quantify the effects of neonicotinoid replacement Flupyradifurone and, the most commonly detected fungicide in free-flying bumblebees, Boscalid, on flying behaviour of bumblebees alone and in combination

## **Chapter 1**

### Advances in insect telemetry

## 1. Chapter 1: Advances in insect telemetry

### 1.1. Introduction

Invertebrates make up approximately 97% of the animal species on Earth (68), with a number of them playing an important role in global food production, human health and wellbeing (6, 10, 24, 69). However, many studies point to a progressive decline of insect species across North America, Europe, and other parts of the world (70). Habitat loss by land use conversion due to intensive agriculture is the main driver of global declines, with agro-chemicals, invasive species, and climate change cited as additional causes (30, 71-73). The recent reports of population declines extend broadly across invertebrate species with losses that could cascade across trophic webs and result in the degradation of ecosystem services (71, 74-78). Understanding how invertebrates respond to these stressors could aid in preventing further population decline and, thus, mitigate ecosystem damage.

Telemetry, defined as the in situ collection of data and automatic transmission to receiving equipment for processing (79), has proved an effective tool for understanding animal

behaviour. Insect telemetry has already helped researchers to understand the detrimental impacts neonicotinoid pesticides, habitat fragmentation, and nutritional stress have on key pollinator species (80-82).

From humble origins in simple visual observations, insect telemetry has evolved to encompass advanced technologies capable of automatically tracking entire colonies of social insects across their lifespans (83). Insect telemetry continues to be an invaluable tool for researchers, but it is not without its limitations and caveats. For example, many techniques rely on the attachment of an electronic tag to the target insect, which may influence normal behaviour and therefore produce inaccurate data (84). Additionally, many telemetry technologies are expensive and require specialist skills for maintenance and function. These factors may limit researchers' decisions and subsequently influence trends in the field of insect telemetry.

In this review, we identify trends in insect telemetry over time. First, we cover a brief history of insect telemetry, looking specifically at three common techniques: radio telemetry, radio-frequency identification (RFID), and harmonic radar. We discuss the development of these technologies, as well as their limitations in this field. Second, we present the results of our systematic review of insect telemetry papers published between 2012 and 2023. We review key findings from radio telemetry, RFID, harmonic radar and 2D barcode studies, and compare the results to the Kissling *et al.* (85) review to provide an overview of trends in insect telemetry. Third, we examine these trends to establish the trajectory of insect telemetry research and the current state of the field. Finally, we speculate on the future role of insect telemetry in research, highlighting potential obstacles and the developing technologies that could circumvent them.

### 1.1.2. History of insect telemetry

Anecdotal evidence exists of Aboriginal hunters tracking bees in New South Wales, Australia. The hunters captured foragers and attached small pieces of feather, spider's web, or grass to a bee's corbiculae (pollen basket). This slowed the bee's flight, facilitating visual tracking and allowing the hunters to locate wild colonies to gather honey and wax (86).

Insect telemetry is an evolution of this early form of insect tracking. To this day, visual observations (albeit often in the form of video recording) remain a valuable research method,

but they present considerable limitations for studying small, fast-moving subjects in their natural environments, especially when monitoring movement across a distance of more than a few metres. The development of video analysis software and improvements to recording equipment eliminated many of those limitations, but it was the advent of automated technologies like RFID, harmonic radar, and radio telemetry which revolutionised research on moving insects. Insect telemetry offers researchers more efficient, more accurate, and longer range options for monitoring insect movement, and has thus become essential to insect movement ecology.

### 1.1.3. A word on tags

A recent review revealed that there currently exist no standard guidelines for assessing how insect telemetry devices impact their target insect (84). Boiteau and Colpitts (87) advised that tag mass should equal less than 33% of a beetle's mass to avoid any deleterious impacts on flight behaviour, but they did not account for impact on aerodynamic relationships nor energetic costs of transporting the tag (see figure 1.1.1 for an example of a tagged insect). Despite this, this guideline has often been extrapolated to other insect species without accounting for interspecies variation.

Beyond Boiteau and Colpitts' guideline, there exist only 'rules of thumb' for tag mass in insect telemetry (e.g. in honeybees [*Apis mellifera*], it is suggested that tag mass should be no more



**Figure 1.1.1:** Attachment of a micro-radio transmitter (300mg) to a male orchid bee *Exaerete frontalis*. (Wikelski et al., 2010).



than the average pollen or nectar load) (88). Accordingly, Batsleer *et al.* (84) suggested that for each new study, the impacts of the specific telemetry technology should be considered in respect to the study species, intraspecific variation, and environmental context relevant to the research question.

Although new telemetric technologies are in development, such as battery-less radio tracking (89), the vast majority of insect telemetry conducted prior to this review uses RFID, harmonic radar, and/or radio telemetry.

#### 1.1.4. Radio telemetry

The first example of wireless telemetry was the radiosonde. Developed in 1930, a radiosonde is a battery-powered transmitter, historically attached to a weather balloon, which transmits meteorological data by radio to a receiver on the ground. Able to transmit data at range, the radiosonde was the predecessor to the wildlife radio telemetry practices developed in the 1960s by Cochran and Lord (90).

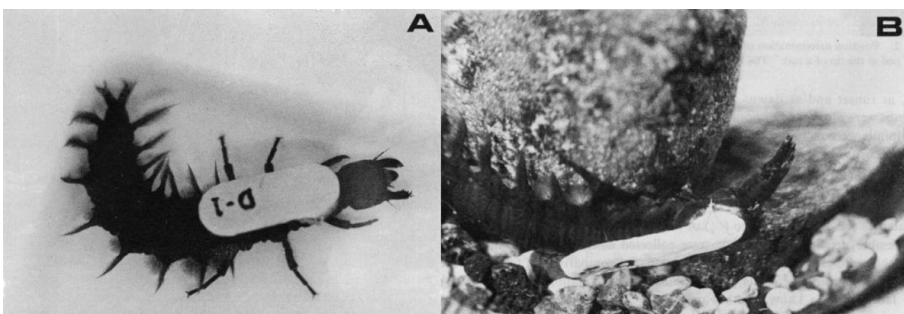
Radio telemetry allows researchers to locate and observe animals in natural environments where visual observations are difficult or disadvantageous. Early in its development, wildlife radio telemetry used Very High Frequency (VHF) transmitters, which were large and heavy and offered limited range. Thus, early research using wildlife radio telemetry was limited to larger animal species.

As technology advanced, transmitter size and mass decreased, and researchers were able to use radio telemetry on insects for the first time. Radio telemetry allows for recording of movement and position data of insects at distances of up to 2km (91). The technology comprises three material components: a transmitter (usually battery-powered), a receiver, and an antenna. A radio signal is emitted by the transmitter (tag), which is affixed to the target organism. This signal is typically in the very high frequency range, or VHF (30-300MHz). The antenna, held by the operator, is rotated until the strongest signal is received. The operator moves towards the signal's origin, checking the direction of the signal frequently, until the tagged animal's location is verified. The signal is transferred to the receiver to be processed and/or stored in a data logger.

In 1988, Hayashi and Nakane (92) used radio telemetry to study *Protohermes grandis*, an aquatic insect whose larvae develop in streambeds. The researchers equipped *P. grandis* larvae with waterproof miniature radio transmitters (figure 1.1.2) and held a small loop antenna 2m above the water's surface to record their positions (figure 1.1.3). They observed that the larvae remained stationary on the streambed for approximately 90% of the experimental period while prey were continuously redistributed by the water current. Thus, radio telemetry facilitated the discovery that *P. grandis* larvae use the ambush method to hunt, and cemented its status as a viable alternative to visual observations.



**Figure 1.1.2:** Position determination of a radio-tagged larva using a small loop antenna equipped at the tip of a rod. This figure has been adopted from (Hayashi and Nakane, 1988) with permission from the publisher.



**Figure 1.1.3:** A dobsonfly larva, *Protohermes grandis*, with a transmitter attached to the back of prothorax. (A) Dorsal view (head capsule width, 7.20 mm). (B) Lateral view. This figure has been adopted from (Hayashi and Nakane, 1988) with permission from the publisher.

Radio telemetry is not, however, without its limitations. The effective range of all radio telemetry devices is determined by the power of the transmitter, i.e. the size of the battery. This necessitates a trade-off between the power and mass of a tag with various consequences across battery life, detection range, and/or subject behaviour (93). Though the impacts of tag mass pose a problem across insect telemetry, they are most noticeable in radio telemetry due to its requirement for heavy active (battery powered) tags. Despite technological advancements across the field, radio telemetry remains on average the heaviest insect telemetry technique (85), and therefore continues to present difficulties for use in research. Thus, the development of lighter passive tags, such as those used in RFID and harmonic radar, constituted a breakthrough in insect telemetry.

#### 1.1.5. RFID

In 1973, Mario Cardullo patented a passive radio transponder with memory. The device, which Cardullo proposed for use in a number of industries, was the predecessor to modern RFID.

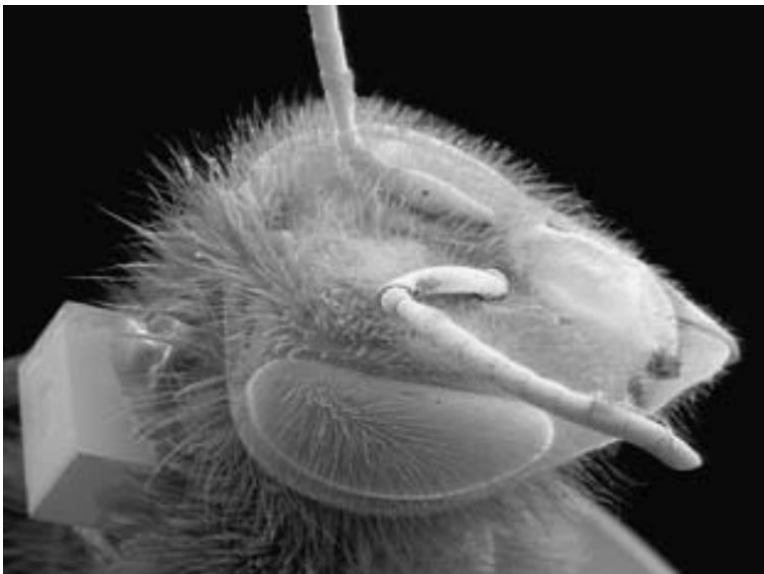
For uses in insect telemetry, RFID technology comprises a passive transponder or “tag” (which uses wireless sensor technology) and a reader (which consists of an antenna and a receiver unit). The reader emits an interrogating electromagnetic signal, which wirelessly powers the RFID tag. In turn, the tag emits a signal, which is received by the reader. The distance over which the reader and tag can communicate effectively depends on several factors, including the power output of the reader, the radio frequency used, and the physical dimensions of the tag (94, 95).

In the 1990s, it became common practice in the EU to use RFID tags on farm animals (injected subcutaneously or attached to the animals’ ears) as a form of individual identification (96, 97). This practice remains important when transporting animals (livestock, zoo animals, and fish), particularly across international borders, i.e. to authenticate country of origin or medical status (98).

The first study to use RFID on insects was conducted by Streit *et al.* in 2003 (95). Due to the relatively small size and weight of the device, the detection range for most RFID tags used in insect telemetry is a few centimetres or millimetres (94, 99). Streit *et al.* compensated for this

shortcoming by developing a tunnel system, which forced tagged bumblebees to pass under the RFID readers when leaving and returning to the nest (95). They used RFID tags on five bumblebees to quantify reader efficiency and ensure the uniquely coded tags could be differentiated from each other. The tags allowed for an individual's exit and arrival times at the nest to be recorded and, thus, the flight duration and foraging activity to be inferred (94).

Unlike radio telemetry and harmonic radar, researchers can feasibly tag and observe entire colonies using RFID. This allows them to quantify colony-wide behaviour and activity throughout the season, making RFID highly suitable for research on social insects (figure 1.1.4). Accordingly, the tunnel system protocol Streit *et al.* (95) pioneered is now common practice when using RFID on social insects, and has been used to study the impacts of parasites, pesticides, viruses, and the weather on the foraging behaviour and activity patterns in honeybee and bumblebee colonies (100-105).



**Figure 1.1.4:** Honeybee (*Apis mellifera*) equipped with a transponder glued to the centre of the dorsal thorax. Adapted from Streit *et al.*, 2003.

#### 1.1.6. Two-dimensional barcodes

A technique that uses lighter tags compared to RFID is the two-dimensional barcode. This method encompasses various names, such as 'optical barcode counter', 'BeeID tags', and

'BEEtag barcode' (106-108). Despite these different names, they function similarly: a camera captures images of the 2D barcode and specialized software identifies and records the position of each uniquely coded barcode (109, 110).

One limitation of RFID technology is its ability to detect tagged insects only when they are in close proximity to the reader. In contrast, two-dimensional barcodes offer the advantage of quantifying individual-level spatial behavior within the nest without requiring a nearby reader. The detection range is determined by the resolution of the camera in use (109). This technique enables the quantification of individual-level variations in both activity and spatial preferences among tagged species. This data can then be used to understand social behaviour as well as within nest behaviour.

One primary advantage of this technique, in contrast to other methods, lies in its independent tag identification for each photo. Consequently, errors do not propagate across frames. For example, tagless automated video tracking relies on information from previous frames for individual tracking (111-113). As such, if an individual is not tracked in one or a few frames (e.g., due to being obscured from the camera's view), the tracking process fails (114).

In addition, this technique is cost-effective, requiring only a camera [Crall et al., (109) used an iPhone 5 camera] and waterproof, tear-resistant paper. The tag tracking software developed by Crall et al. (109) is open source, freely available, and hosted on GitHub. Furthermore, this technique does not require a homogeneous background, distinguishing it from previous tagless video tracking methods (112, 114).

However, this system is not without its' disadvantages. Similar to many tracking techniques involving tagging, this method may induce stress in individuals during both handling (115) and tag application (116). Adequate lighting is essential since this system relies on the recognition of tags in the images (109). Finally, managing and processing a substantial volume of data, especially when dealing with long-term data that includes a high number of images for the software to process, can be computationally taxing.

Overall, the two-dimensional barcode system offers a valuable tool for the quantification of individual-level spatial behaviors, with the potential to advance our understanding of social and nest behaviors among social insect species.

### 1.1.7. Harmonic Radar

Due to their short detection range, RFID and 2D barcodes cannot provide spatial and temporal data for free-flying insects, and this limits their use in research on insect movement. However, harmonic radar technology offers the long detection range of radio telemetry with the low mass tags of RFID, and thus broadened the scope of research potential in the field of insect telemetry (117).

Though radar had been used to observe the flight of insects for many years before the invention of the harmonic radar, low altitude data proved difficult to obtain due to interference or “clutter” from ground features (118). Harmonic radar technology reduces radar clutter by introducing passive transponders known as harmonic transponders.

A harmonic radar comprises a harmonic transponder or “tag”, a transmitter, and a receiver (figure 1.1.5). Capable of tracking the position of tagged insects up to a range of approximately 500m (52, 119, 120), a harmonic radar functions by transmitting a signal which the tag re-transmits at a harmonic frequency (usually the second harmonic of the original signal, i.e. half the wavelength of the initial frequency). The receiver is tuned to this harmonic frequency, and therefore only receives transmissions from the tag, thereby reducing clutter from environmental reflections.

In an early trial of the harmonic radar, Riley *et al.* tagged and tracked bumblebee and honeybee foragers from their nests to a plot of *Phacelia tanacetifolia* and vice versa over a distance of 50-250m (118). For the first time, it was possible to track and quantify the flight path of foraging bees, and accurately estimate their air speed. Following on from this preliminary work, Capaldi *et al.* (88) used the harmonic radar to study the flight behaviour of honeybees (specifically the orientation flight) in the first published research using harmonic radar on flying insects.

Since then, the harmonic radar has proven beneficial for unravelling the navigational techniques and movement behaviours of a number of volant insect species (121-124). However, the technology has limitations. For example, the tagged insect cannot be detected



*Figure 1.1.5: Scanning harmonic radar from Rothamsted Research, Hertfordshire, UK. Photo credit: Thomas R. Oliver.*

outside of the radar horizon (i.e. on the ground or at high altitudes (125)) nor when within the radar shadow cast by large objects such as trees or buildings (117). In addition, a limited number of tagged individuals can be tracked at the same time, due to the transponders lacking the ability to re-emit a unique signal. Furthermore, like in all insect telemetry techniques, the size and mass of the tag may impact the behaviour and flight capabilities of the tagged insect, and thus limits the application of harmonic radar technology to smaller species.

## 1.2. Methods

### 1.2.1. Systematic Literature Search and Strategy Applied

I conducted a literature search in the *Web of Science* (WoS) database across all journals. The search was conducted in English only and was restricted from October 2012 to the time the review was conducted (February 2023). Literature published prior to October 2012 was not reviewed, having been covered previously in a review on the progression of the field of insect telemetry from the late 1980s to October 2012 (85).

To formulate my search and screening protocols, I followed the guidelines of the Collaboration for Environmental Evidence (126) to ensure transparency and repeatability for my review. I used multiple search terms (see Table 1.2.1) to ensure all potentially relevant publications were included in the review. I excluded a number of irrelevant categories during the searching process (see supplemental information S1.1).

**Table 1.2.1:** The Boolean string of search terms used to search the Web of Science database. Each keyword within each topic was separated by the Boolean "OR" while the topics were separated by the Boolean "AND". \* indicates a wildcard and terms in quotation marks ("") are considered in the search as one word.

Topics		
Invertebrate		Telemetry
Acanaloniidae	Leptoxyphidae	"Animal tracking"
Acanthocnemidae	Leptomantellidae	"Flight path"
Acanthopidae	Leptophlebiidae	"Habitat selection"
Acanthopteroctetidae	Leptopodidae	"Radiotracking"
Acanthosomatidae	Leptopsyllidae	"Habitat use radiotracking"
Acartophthalmidae	Lestidae	"Harmonic Radar"
Achilidae	Lestoideidae	"Insect telemetry"
Achilixiidae	Lestoniidae	"Invertebrate tracking"
Acleridae	Leucospidae	"Radio frequency identification tag"
Acrididae	Leuctridae	"Radio telemetry*"
Acroceridae	Liadopsyllidae	"Radio tracking home range"
Acrolophinae	Libellulidae	"Search pattern*"
Adelgidae	Lice*	"Tracking home range movements"
Adelidae	Limacodidae	Movement*
Aderidae	Limnephilidae	Radiotelemetry*
Aenictopecheidae	Limnichidae	Radiotracking
Aenigmatineidae	Limoniidae	RFID
Aenigmatineidae	Lindeniidae	
Aeolothripidae	Linognathidae	



Aeshnidae	Liopteridae	Telemetry
Aetalionidae	Liposcelididae	Tracking
Agaonidae	Lithidiidae	Trajectory
Agapythidae	Liturgusidae	
Agathemeridae	Liviidae	
Agathiphagidae	Locust*	
Agromyzidae	Lonchaeidae	
Agyrtidae	Lonchopteridae	
Aididae	Lophocateridae	
Akalyptoischiidae	Lophocoronidae	
Alderfl*	Lophopidae	
Alexiidae	Lucanidae	
Aleyrodidae	Lutrochidae	
Alucitidae	Lycaenidae	
Alydidae	Lycidae	
Amanipodagrionidae	Lyctocoridae	
Ameletidae	Lygaeidae	
Ameletopsidae	Lygistorrhinidae	
Amelidae	Lymexylidae	
Ametropodidae	Lypusidae	
Amorphoscelidae	Maamingidae	
Amphientomidae	Machaerotidae	
Amphipsocidae	Machilidae	
Amphipterygidae	Macromiidae	
Amphizoidae	Macropiratidae	
Ampulicidae	Macroveliidae	
Anamorphidae	Maindroniidae	
Anaplectidae	Majangidae	
Ancistropsyllidae	Malcidae	
Andesembiidae	Manicapsocidae	
Andesianidae	Manti*	
Andrenidae	Mantidae	
Angelidae	Mantispidae	
Anisacanthidae	Mantoididae	
Anisembiidae	Mantophasmatidae	
Anisolabididae	Margarodidae	
Anisopodidae	Marginidae	
Anomalopsychidae	Mastotermitidae	
Anomoeotidae	Matsucoccidae	
Anomosetidae	Mauroniscidae	
Anostomatidae	Mayfl*	
Ant*	Meenoplidae	
Anthelidae	Meessiidae	
Anthicidae	Megachilidae	
Anthocoridae	Megalodontesidae	
Anthomyiidae	Megalopodidae	
Anthomyzidae	Megalopygidae	
Anthribidae	Megalyridae	
Antipodoeciidae	Megapodagrionidae	
Apachyidae	Megarididae	

Apataniidae	Megastigmidae	
Apatelodidae	Meinertellidae	
Aphalaridae	Melandryidae	
Aphelinidae	Melanemerellidae	
Aphid*	Melittidae	
Aphididae	Melizoderidae	
Aphrophoridae	Meloidae	
Apidae	Melyridae	
Apioceridae	Membracidae	
Apsilocephalidae	Menoponidae	
Apteropanorpidae	Meropeidae	
Apystomyiidae	Merothripidae	
Aradidae	Meruidae	
Archembiidae	Meruidae	
Archeocrypticidae	Mesagrionidae	
Archipsocidae	Meschiidae	
Archotermopsidae	Mesembrinellidae	
Arctopsychidae	Mesopsocidae	
Argidae	Mesoveliidae	
Argiolestidae	Metallyticidae	
Argyresthiidae	Metarbelidae	
Arixeniidae	Metaxinidae	
Arrhenophaninae	Micrococcidae	
Artematopodidae	Micromalthidae	
Artheneidae	Micromalthidae	
Ascalaphidae	Micropezidae	
Aschiphasmaticidae	Microphysidae	
Asilidae	Micropterigidae	
Asiopsocidae	Milichiidae	
Aspidytidae	Millieriidae	
Asteiidae	Miridae	
Asterolecaniidae	Mnesarchaeidae	
Atelestidae	Mogoplistidae	
Athericidae	Molannidae	
Atriplectididae	Momphidae	
Attelabidae	Monophlebidae	
Attevidae	Monotomidae	
Aulacigastridae	Mordellidae	
Austrolembiidae	Mormotomyiidae	
Australimyziidae	Moth*	
Austrocorduliidae	Murmidiidae	
Austroleptidae	Musapsocidae	
Austroperlidae	Muscidae	
Austropetaliidae	Mutillidae	
Autostichidae	Mycetaeidae	
Axymyiidae	Mycetophagidae	
Azotidae	Mycetophilidae	
Bacillidae	Mycteridae	
Baetidae	Mydidae	
Baetiscidae	Myerslopiidae	

Bahiaxenidae	Mymaridae	
Baissoferidae	Mymarommatidae	
Barklice*	Myopsocidae	
Batrachedridae	Myraboliidae	
Bedelliidae	Myrmecolacidae	
Bee*	Myrmecophilidae	
Beesoniidae	Myrmeleontidae	
Beetle*	Myrmosidae	
Behningiidae	Mystacinobiidae	
Belidae	Mythicomyiidae	
Belohinidae	Nabidae	
Belostomatidae	Nannochoristidae	
Beraeidae	Nannodastiidae	
Berothidae	Nanomantidae	
Berytidae	Natalimyza	
Bethylidae	Natalimyziidae	
Bibionidae	Naucoridae	
Biphyllidae	Nemestrinidae	
Bittacidae	Nemonychidae	
Blaberidae	Nemopteridae	
Blasticotomidae	Nemouridae	
Blastobasidae	Neophemeridae	
Blattidae	Neopetaliidae	
Blephariceridae	Neopseustidae	
Blissidae	Neotheoridae	
Boganiidae	Neotheoridae	
Bohartillidae	Nepidae	
Bolboceratidae	Neriidae	
Bolbomyiidae	Nesameletidae	
Bolitophilidae	Nevrorthidae	
Bombycidae	Nicoletiidae	
Bombyliidae	Ninidae	
Boopidae	Nitidulidae	
Boreidae	Nixoniiidae	
Boridae	Nocticolidae	
Bostrichidae	Noctuidae	
Bothrideridae	Nogodinidae	
Brachodidae	Nolidae	
Brachycentridae	Nosodendridae	
Brachyceridae	Noteridae	
Brachypsectridae	Nothybus	
Brachytronidae	Notodontidae	
Braconidae	Notonectidae	
Bradynobaenidae	Notonemouridae	
Brahmaeidae	Nycteribiidae	
Braulidae	Nymphalidae	
Brentidae	Nymphidae	
Bryopsocidae	Nymphomyiidae	
Bucculatricidae	Ochodaeidae	
Bug*	Ochteridae	

Buprestidae	Odiniidae	
Butterfl*	Odontoceridae	
Byrrhidae	Oeconesidae	
Byturidae	Oecophoridae	
Caddisfl*	Oedemeridae	
Caeciliusidae	Oenosandridae	
Caenidae	Oestridae	
Calamoceratidae	Oligoneuriidae	
Caliscelidae	Oligotomidae	
Callaphididae	Omethidae	
Callidulidae	Ommatidae	
Calliphoridae	Ommexechidae	
Callirhipidae	Oniscigastridae	
Calophyidae	Opetiidae	
Calopsocidae	Opomyzidae	
Calopterygidae	Opostegidae	
Camillidae	Oreogetonidae	
Campichoetidae	Oreoleptidae	
Canacidae	Ormyridae	
Canopidae	Orsodacnidae	
Cantharidae	Ortheziidae	
Canthyloscelidae	Orussidae	
Capniidae	Osmylidae	
Carabidae	Oxycarenidae	
Carayonemidae	Oxypeltidae	
Caridae	Pachygronthidae	
Carnidae	Pachyneuridae	
Carsidaridae	Pachytroctidae	
Carthaeidae	Palaeosetidae	
Castniidae	Palaephatidae	
Cavognathidae	Palingeniidae	
Cecidomyiidae	Pallopteridae	
Cecidosidae	Pamphagidae	
Celyphidae	Pamphagodidae	
Cerambycidae	Pamphiliidae	
Ceraphronidae	Panorpidae	
Ceratocombidae	Panorpodidae	
Ceratophyllidae	Pantophthalmidae	
Ceratopogonidae	Papilionidae	
Cercopidae	Paraleucopidae	
Cerococcidae	Parastrachiidae	
Cerophytidae	Passalidae	
Cerylonidae	Passandridae	
Chaeteessidae	Pediciidae	
Chaetosomatidae	Pedicinidae	
Chalcididae	Pediculidae	
Chalcodryidae	Pelecinae	
Chamaemyiidae	Pelecorhynchidae	
Chaoboridae	Peloriidae	
Chathamidae	Peltidae	

Chelisochidae	Peltoperlidae	
Chelonariidae	Pentatomidae	
Chironomidae	Pergidae	
Chlorocyphidae	Perilampidae	
Chlorogomphidae	Perilestidae	
Chloroperlidae	Peripsocidae	
Chloropidae	Periscelididae	
Choreutidae	Perissommatidae	
Choristidae	Perlidae	
Chorotypidae	Perlodidae	
Chroicopteridae	Permocupedidae	
Chrysididae	Petaluridae	
Chrysomelidae	Phacopteronidae	
Chrysopidae	Phaeomyiidae	
Chyphotidae	Phalacridae	
Chyromyidae	Phalangopsidae	
Cicada*	Phasmatidae	
Cicadellidae	Phaudidae	
Cicadidae	Phenacoleachiidae	
Ciidae	Phengodidae	
Cimbicidae	Phiditiidae	
Cimeliidae	Philogangidae	
Cimicidae	Philogeniidae	
Cixiidae	Philopotamidae	
Cladiopsocidae	Phlopteridae	
Clambidae	Philorheithridae	
Clastopteridae	Philosinidae	
Cleridae	Philotarsidae	
Clothodidae	Phlaeothripidae	
Clusiidae	Phloeidae	
Cneoglossidae	Phloeostichidae	
Coccidae	Phloiophilidae	
Coccinellidae	Phoenicococcidae	
Cockroach*	Phoridae	
Coelopidae	Photinaidae	
Coelostomiidae	Phryganeidae	
Coenagrionidae	Phycosecidae	
Coleophoridae	Phylliidae	
Colletidae	Phylloxeridae	
Colobathristidae	Pieridae	
Coloburiscidae	Piesmatidae	
Compsocidae	Piophilidae	
Conchaspidae	Pipunculidae	
Coniopterygidae	Pirenidae	
Conopidae	Pisuliidae	
Cooloolidae	Pityococcidae	
Coptopterygidae	Planthopper*	
Cordulegastridae	Plataspidae	
Cordulephyidae	Platycnemididae	
Corduliidae	Platygastridae	

Coreidae	Platypezidae	
Corethrellidae	Platystictidae	
Corioxenidae	Platystomatidae	
Corixidae	Plectrotarsidae	
Corydalidae	Pleidae	
Corydiidae	Pleocomidae	
Corylophidae	Pneumoridae	
Cosmopterigidae	Podabrocephalidae	
Cossidae	Polleniidae	
Crabronidae	Polycentropodidae	
Crambidae	Polyctenidae	
Cremifaniidae	Polymitarcyidae	
Cricket*	Polyplacidae	
Crowsoniellidae	Polythoridae	
Cryptocercidae	Pompilidae	
Cryptochetidae	Potamanthidae	
Cryptophagidae	Praydidae	
Cryptorhamphidae	Priasilphidae	
Ctenophthalmidae	Prionoceridae	
Ctenostylidae	Prionoglaridae	
Cucujidae	Prisopodidae	
Culicidae	Proctotrupidae	
Cupedidae	Prodidactidae	
Curaliidae	Prodoxidae	
Curculionidae	Promastacidae	
Curtonotidae	Propalticidae	
Cybocephalidae	Prophalangopsidae	
Cyclaxyridae	Proscopiidae	
Cyclotornidae	Prosopistomatidae	
Cydniidae	Prostomidae	
Cylindrachetidae	Protocucujidae	
Cylindrotomidae	Protolestidae	
Cymidae	Protoneuridae	
Cynipidae	Protopeltidae	
Cypselosomatidae	Prototheoridae	
Dactylopiidae	Protrinemuridae	
Dactylopterygidae	Psephenidae	
Dalceridae	Pseudobistonidae	
Damasippoididae	Pseudocaeciliidae	
Damselfl*	Pseudococcidae	
Dascillidae	Pseudocorduliidae	
Dasydemellidae	Pseudolestidae	
Decliniidae	Pseudophasmatidae	
Delphacidae	Pseudopomyzidae	
Depressariidae	Pseudostigmatidae	
Derbidae	Psilidae	
Dericorythidae	Psilopsocidae	
Dermestidae	Psocidae	
Derodontidae	Psychidae	
Deroplatyidae	Psychodidae	

Deuterophlebiidae	Psychomyiidae	
Devadattidae	Psychopsidae	
Diadocidiidae	Psyllidae	
Diamphipnoidae	Pterogeniidae	
Diapheromeridae	Pterolonchidae	
Diapriidae	Pteromalidae	
Diaspididae	Pteronarcyidae	
Diastatidae	Pterophoridae	
Dicteriidae	Pthiridae	
Dictyopharidae	Ptiliidae	
Dilaridae	Ptilodactylidae	
Dinidoridae	Ptiloneuridae	
Diopsidae	Ptinidae	
Diphyllostomatidae	Ptychopteridae	
Diplatyidae	Pulicidae	
Diprionidae	Putoidae	
Dipseudopsidae	Pygidicranidae	
Dipsocoridae	Pyralidae	
Discolomatidae	Pyrgacrididae	
Disteniidae	Pyrgomorphidae	
Ditomyiidae	Pyrgotidae	
Dixidae	Pyrochroidae	
Dobsonfl*	Pyrrhocoridae	
Doidae	Pythidae	
Dolabellopsocidae	Ragadidae	
Dolichopodidae	Rangomaramidae	
Douglasiidae	Raphidiidae	
Dragonfl*	Reduviidae	
Drepanidae	Rhachiberothidae	
Drosophilidae	Rhadalidae	
Dryinidae	Rhagionidae	
Dryomyzidae	Rhagophthalmidae	
Dryopidae	Rhaphidophoridae	
Dudgeoneidae	Rhiniidae	
Dytiscidae	Rhinophoridae	
Earwig*	Rhinotermitidae	
Echinophthiriidae	Rhipiceridae	
Ecnomidae	Rhipidolestidae	
Ectobiidae	Rhopalidae	
Ectopsocidae	Rhopalosomatidae	
Elachistidae	Rhyacophilidae	
Elateridae	Rhyarochromidae	
Elenchidae	Richardiidae	
Elipsocidae	Ricinidae	
Elmidae	Rimanellidae	
Embiidae	Riodinidae	
Embolemidae	Ripiphoridae	
Empididae	Ripiphoridae	
Empusidae	Ripterygidae	
Encyrtidae	Rivetinidae	

Endecatomiidae	"Rock bristletail*"	
Enderleinellidae	Roeslerstammiidae	
Endomychidae	Romaleidae	
Endromidae	Ropalomeridae	
Enicocephalidae	Rotoitidae	
Eomeropidae	Saileriolidae	
Epaphroditidae	Saldidae	
Epermeniidae	Salpingidae	
Ephemerellidae	Sapygidae	
Ephemeridae	Sarcophagidae	
Ephydriidae	Saturniidae	
Epicopeiidae	Sawfl*	
Epimarptidae	Scarabaeidae	
Epimetopidae	Scathophagidae	
Epipsocidae	Scatopsidae	
Epipygidae	Scelembiidae	
Epipyropidae	Scelionidae	
Erebidae	Scenopinidae	
Eremiaphilidae	Schistonoeidae	
Eremochaetidae	Schizodactylidae	
Eriococcidae	Schizopodidae	
Eriocottidae	Schreckensteiniidae	
Eriocraniidae	Sciaridae	
Erirhinidae	Sciomyzidae	
Erotylidae	Scirtidae	
Eucharitidae	Sclerogibbidae	
Eucinetidae	Scolebythidae	
Eucnemidae	Scoliidae	
Eulichadidae	Scorpionfl*	
Eulophidae	Scraptiidae	
Eumastacidae	Scutelleridae	
Eupelmidae	Scythruidae	
Euphaeidae	Sematuridae	
Eupsilobiidae	Sepsidae	
Eupterotidae	Sericostomatidae	
Eurybrachidae	Serritermitidae	
Eurytomidae	Sesiidae	
Eustheniidae	Sialidae	
Euthyplociidae	Sierolomorphidae	
Euxestidae	Signiphoridae	
Evaniidae	Silphidae	
Evocoidae	Silvanidae	
Fanniidae	Silverfish*	
Fergusoninidae	Simaethistidae	
Figitidae	Simuliidae	
Flatidae	Sinopyrophoridae	
Flea*	Siphonuridae	
Flies*	Siphuriscidae	
Fly*	Siricidae	
Forficulidae	Sisyridae	



Formicidae	Smicripidae	
Froghopper*	Snakefl*	
Fulgoridae	Socialidae	
Galacticidae	Somabrachyidae	
Galinthiidae	Somatiidae	
Gelastocoridae	Sparasionidae	
Gelechiidae	Spercheidae	
Gengidae	Sphaeritidae	
Geocoridae	Sphaeriusidae	
Geometridae	Sphaeroceridae	
Georissidae	Sphaeropsocidae	
Geoscelionidae	Sphecidae	
Geotrupidae	Sphindidae	
Gerridae	Spittlebug*	
Glaphyridae	Spongiphoridae	
Glaresidae	Staphylinidae	
Glossinidae	Stathmopodidae	
Glossosomatidae	Steingeliidae	
Glyphipterigidae	Stemmocryptidae	
Gobryidae	Stenocephalidae	
Goeridae	Stenomicridae	
Gomphidae	Stenopelmatidae	
Gomphomacromiidae	Stenophyllidae	
Gonypetidae	Stenopsocidae	
Gracillariidae	Stenopsychidae	
Grasshopper*	Stenotrachelidae	
Gripopterygidae	Stenotritidae	
Gryllacrididae	Stephanidae	
Gryllidae	Stephanocircidae	
Grylloblattidae	Stictococcidae	
Gryllotalpidae	"Stick insect**"	
Gyrinidae	Stigmacoccidae	
Gyropidae	Stolotermitidae	
Haaniidae	Stonefl*	
Haematomyzidae	Stratiomyidae	
Haematopinidae	Streblidae	
Halictidae	Strongylophthalmyiidae	
Halictophagidae	Styloperlidae	
Halimococcidae	Stylopidae	
Haliplidae	Stylotermitidae	
Hebridae	Synchroidae	
Hectopsylla	Synlestidae	
Hectopsyllidae	Syntelliidae	
Hedyidae	Synthemistidae	
Helcomyzidae	Syringogastridae	
Helicopsychidae	Syrphidae	
Heliozelidae	Tabanidae	
Helophoridae	Tachinidae	
Helosciomyzidae	Taeniopterygidae	
Helotidae	Taldycupedidae	

Helotrephidae	Tanaoceridae	
Hemerobiidae	Tanaostigmatidae	
Hemimeridae	Tanyderidae	
Hemiphlebiidae	Tanypezidae	
Hemipsocidae	Tasimiidae	
Hepialidae	Tasmosalpingidae	
Heptageniidae	Tatocnemididae	
Hermatobatidae	Telephlebiidae	
Hesperiidae	Teloganodidae	
Hesperinidae	Tenebrionidae	
Hesperinus	Tenthredinidae	
Heteragrionidae	Tephritidae	
Heterobathmiidae	Teratemiidae	
Heteroceridae	Teratomyzidae	
Heterocheilidae	Teredidae	
Heterogastridae	Termitaphididae	
Heterogynaidae	Termite*	
Heterogynidae	Termitidae	
Heteronemiidae	Tessaratomidae	
Heteropterygidae	Tethepomyiidae	
Heterothripidae	Tetracampidae	
Hilarimorphidae	Tetatomidae	
Himantopteridae	Tetrigidae	
Hippoboscidae	Tettigarctidae	
Histeridae	Tettigometridae	
Hobartiidae	Tettigoniidae	
Hodotermitidae	Thanerocleridae	
Hodotermopsidae	Thaumaleidae	
Hodotermopsis	Thaumastellidae	
Homalocnemididae	Thaumastellidae	
Homotomidae	Thaumastocoridae	
Hoplocoryphidae	Thaumatonneuridae	
Hoplopleuridae	Therevidae	
Huttoninidae	Thericleidae	
Hyblaeidae	Thespidae	
Hybosoridae	Thrip*	
Hybotidae	Thripidae	
Hydraenidae	Throscidae	
Hydrobiosidae	Thymalidae	
Hydrochidae	Thynnidae	
Hydrometridae	Thyreocoridae	
Hydrophilidae	Thyrididae	
Hydropsychidae	"Tiger beetle"	
Hydroptilidae	Tineidae	
Hydroscaphidae	Tingidae	
Hygrobiiidae	Tiphiidae	
Hymenopodidae	Tipulidae	
Hyocephalidae	Tischeriidae	
Hypochthonellidae	Torridincolidae	
Hypolestidae	Tortricidae	

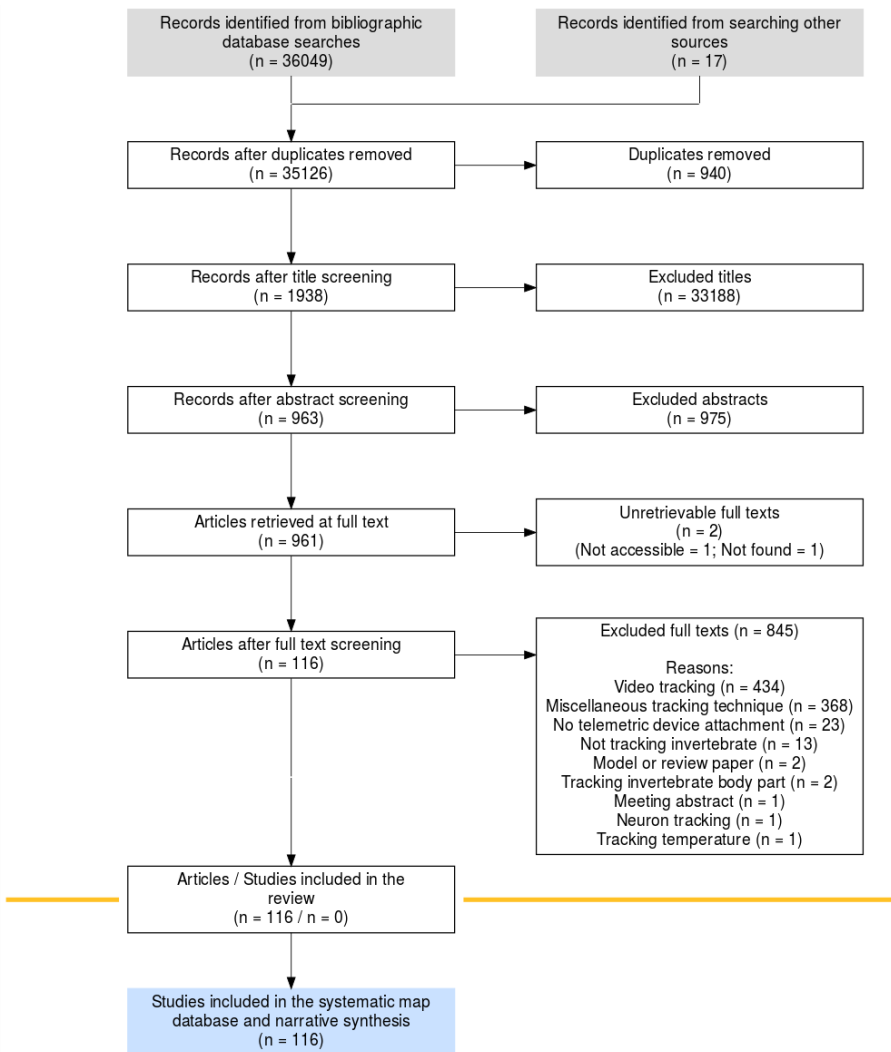
Hysipterygidae	Torymidae	
Hystrichopsyllidae	Toxoderidae	
Ibaliidae	Trachelostenus	
Iberobaeniidae	Trachypachidae	
Icebug*	Trachypetinae	
Ichneumonidae	Treehopper*	
Immidae	Trichoceridae	
Inbiomyiidae	Trichodectidae	
Incurvariidae	Trichogrammatidae	
Inocelliidae	Trichopsocidae	
Insect*	Tricorythidae	
Invertebrate*	Trictenotomidae	
Iridopterygidae	Tridactylidae	
Ironomyiidae	Tridentaformidae	
Ischnopsyllidae	Trigonalidae	
Ismaridae	Trigonopterygidae	
Isonychiidae	Trimenoponidae	
Isostictidae	Triozidae	
Issidae	Tristiridae	
Ithonidae	Troctopsocidae	
Jacobsoniidae	Trogidae	
Janzenellidae	Trogossitidae	
Jurasidae	Tropiduchidae	
Jurodidae	Tryonicidae	
Kalophryganeidae	Uenoidae	
Kalotermitidae	Uliidae	
Karschiellidae	Ulodidae	
Kateretidae	Ulurumiidae	
Kermesidae	Uraniidae	
Keroplastidae	Urodidae	
Kerriidae	Urostylididae	
Kinnaridae	Ustyurtiidae	
Kokiriidae	Uzelothripidae	
Kuwaniidae	Valeseguyidae	
Labiduridae	Vanhorniidae	
Lacewing*	Veliidae	
Lachesillidae	Vermileonidae	
Lacturidae	Vesperidae	
Laemobothiidae	Vespidae	
Laemophloeidae	Vietnamellidae	
Lamingtoniidae	Wasp*	
Lamproblattidae	"Water treader*"	
Lampyridae	Webspinner*	
Largidae	Weevil*	
Larv*	Whalleyanidae	
Lasiocampidae	Xenasteiidae	
Lathiceridae	Xiphocentronidae	
Latridiidae	Xiphydriidae	
Lauxaniidae	Xyelidae	
Lecanodiaspididae	Xylococcidae	

Lecithoceridae	Xylomyidae	
Leiodidae	Xylophagidae	
Lentulidae	Xyloryctidae	
Lepiceridae	Yponomeutidae	
Lepidopsocidae	Ypsolophidae	
Lepidostomatidae	Zopheridae	
Lepidotrichidae	Zorotypidae	
Lepismatidae	Zygaenidae	
Leptoceridae		

### 1.2.2. Eligibility criteria and screening

One researcher (T.R.O) performed the screening process and two other researchers (P.C & A.M.R) conducted a 10% sample of screening at each step. The discrepancy rate between researchers at each step was <10%.

Our screening process is visualised in Figure 1.2.1 (126).



**Figure 1.2.1:** ROSEs (RepOrting standards for Systematic Evidence Syntheses) protocol flow chart visualising the screening process for papers included in the systematic map.

### 1.3. Results

Here I provide an overview of insect telemetry studies published between October 2012 and February 2023. I identified 116 insect telemetry studies from the *Web of Science* database (WoS) (figure 1.2.1) following the ROSEs systematic search strategy guidelines (126). The identified papers were characterised by the telemetry technology used: radio telemetry, RFID, harmonic radar, and 2D barcodes (Tables 1.3.1, 1.3.2, 1.3.3, and 1.3.4 respectively).

I identified 434 studies using video footage and an additional 368 using miscellaneous telemetry technologies such as visual observations. I excluded these studies from the final total, as I was only interested in studies involving the attachment of tags to the target insect species. I explicitly avoided including unpublished reports or theses in the study as these are frequently difficult to access.

**Table 1.3.1:** Summary of case studies using Radio Telemetry (N=33). (N= 38 when papers with more than one study species are accounted for). The aims of each study (Yes/No) are summarised within six categories: FOR, foraging behaviour; MOV, movement distance; HAB, habitat use and selection; ; IMP, impact of tracking technology; NAV, navigation; EFF, efficacy of telemetry technology; SOC, social behaviour; DIS, dispersal; MIG, migration.

Insect characteristics			Study aims										Maximum range (m)	Case study
Species	Family	Flying	FOR	MOV	HAB	IMP	NAV	EFF	SOC	DIS	MIG			
Rhynchophorus ferrugineus	Curculionidae	Y	N	N	N	N	N	Y	Y	Y	N	90	Al Ansi <i>et al.</i> (2020)	
Eurycantha calcarata	Lonchodidae	N	N	N	N	N	N	Y	N	Y	N	50	Boisseau <i>et al.</i> (2020)	
Eurycantha insularis	Lonchodidae	N	N	N	N	N	N	Y	N	Y	N	50	Boisseau <i>et al.</i> (2020)	
Bombus pauloensis	Apidae	Y	N	N	N	N	N	Y	Y	N	N	200	Cavigliasso <i>et al.</i> (2020)	
Bombus pauloensis	Apidae	Y	N	N	N	N	N	Y	Y	N	N	200	Cavigliasso <i>et al.</i> (2020)	
Osmoderma eremita	Scarabaeidae	Y	N	N	Y	N	N	Y	N	N	N	250	Chiari <i>et al.</i> (2019)	
Cerambyx cerdo	Cerambycidae	Y	N	N	Y	N	N	N	Y	Y	N	250	Drag & Orzek (2018)	
Carabus coriaceus	Carabidae	N	N	N	N	N	N	Y	Y	N	N	60	Elek <i>et al.</i> (2021)	
Danaus plexippus	Nymphalidae	Y	N	N	N	N	N	Y	Y	Y	N	135	Fischer <i>et al.</i> (2020)	
Danaus plexippus	Nymphalidae	Y	N	N	N	N	N	N	N	N	Y	150	Fischer <i>et al.</i> (2021)	
Danaus plexippus	Nymphalidae	Y	Y	N	N	N	N	Y	Y	Y	N	135	Fisher & Bradbury (2022)	
Hemiteles crassidens	Anostomatidae	N	N	N	N	N	N	Y	N	Y	N	500	Gwynne & Kelly (2018)	
Triatoma gerstaeckeri	Reduviidae	Y	N	N	N	N	N	Y	Y	N	N	20	Hamer <i>et al.</i> (2018)	
Rhynchophorus ferrugineus	Dryophthoridae	Y	N	N	N	N	N	N	N	Y	N	NA	Hamidi <i>et al.</i> (2017)	
Vespa velutina	Vespidae	Y	N	N	N	N	N	N	Y	Y	Y	817	Kennedy, <i>et al.</i> (2018)	
Allomyrina dichotoma	Scarabaeidae	Y	N	N	N	N	N	N	Y	Y	N	80	Kim <i>et al.</i> (2022)	
Dorcus titanus	Lucanidae	Y	N	N	N	N	N	N	Y	Y	Y	80	Kim <i>et al.</i> (2022)	
Danaus plexippus	Nymphalidae	Y	N	N	N	N	Y	N	Y	N	N	1000	Knight <i>et al.</i> (2019)	
Anax junius	Aeshnidae	Y	N	N	N	N	Y	N	Y	N	N	1000	Knight <i>et al.</i> (2019)	
Paysandisia archon	Castniidae	Y	N	N	N	N	N	Y	Y	Y	N	500	Liégeois <i>et al.</i> (2016)	
Trypoxylus dichotomus	Scarabaeidae	Y	N	N	N	N	N	N	Y	Y	Y	800	McCullough (2013)	
Anax imperator	Aeshnidae	Y	N	N	N	N	N	Y	Y	N	N	200	Minot <i>et al.</i> (2021)	
Oryctes rhinoceros	Scarabaeidae	Y	N	N	N	N	N	Y	Y	N	N	500	Moore <i>et al.</i> (2017)	
Cordulegaster erronea	Cordulegastriidae	Y	N	N	N	N	N	Y	Y	N	N	180	Moskowitz & May (2017)	
Carabus olympiae	Carabidae	N	N	N	N	N	N	Y	Y	N	N	300	Negro <i>et al.</i> (2017)	
Carabus hungaricus	Carabidae	N	N	N	N	N	N	Y	Y	N	N	120m	Růžicková & Bérces (2019)	
Carabus ulrichii	Carabidae	N	N	N	N	N	N	Y	Y	N	N	60	Růžicková & Veselý (2016)	
Carabus ulrichii	Carabidae	N	N	N	N	N	N	N	Y	Y	N	60	Růžicková & Veselý (2016)	
Carabus coriaceus	Carabidae	N	N	N	N	N	N	Y	N	N	N	60	Růžicková & Veselý (2018)	
Carabus coriaceus	Carabidae	N	N	N	N	N	N	Y	N	N	N	60	Růžicková <i>et al.</i> (2021)	
Anabrus simplex	Tettigoniidae	N	N	N	N	N	Y	N	N	N	N	500	Srygley & Lorch (2013)	
Anabrus simplex	Tettigoniidae	N	N	N	N	N	Y	N	N	N	N	500	Srygley & Lorch (2016)	
Lucanus cervus	Lucanidae	Y	N	N	N	N	N	Y	Y	N	N	250	Thomas <i>et al.</i> (2018)	
Lucanus cervus	Lucanidae	Y	N	N	N	N	Y	N	N	N	N	100	Tini <i>et al.</i> (2017a)	
Lucanus cervus	Lucanidae	Y	N	N	N	N	N	Y	N	N	N	100	Tini <i>et al.</i> (2017b)	
Lucanus cervus	Lucanidae	Y	N	N	Y	N	N	Y	N	N	N	100	Tini <i>et al.</i> (2018)	
Troides aeneus	Papilionidae	Y	N	N	Y	N	N	Y	Y	N	N	1500	Wang <i>et al.</i> (2019)	
Danaus plexippus	Nymphalidae	Y	N	N	N	N	Y	N	N	N	N	1000	Wilcox <i>et al.</i> (2021)	

**Table 1.3.2:** Summary of case studies using RFID (N=37). (N=41 when papers with more than one study species are accounted for).

Insect characteristics			Study aims									Case study
Species	Family	Flying	FOR	MOV	HAB	IMP	NAV	EFF	SOC	DIS	MIG	
<i>Apis mellifera</i>	Apidae	Y	N	N	N	N	N	N	N	N	Y	Al & Takahashi. (2021).
<i>Bombus impatiens</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Avila et al. (2022)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Ayup et al. (2021)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Benaets et al. (2017)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	Y	N	N	N	N	N	Beyaert et al. (2012)
<i>Apis mellifera</i>	Apidae	Y	N	N	N	Y	N	N	N	N	N	Capela et al. (2022)
<i>Cosmopolites sordidus</i>	Curculionidae	N	N	Y	N	N	N	N	N	N	N	Carval et al. (2015)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Collin et al. (2019)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Collin et al. (2022)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Collin et al. (2022)
<i>Melipona seminigra</i>	Apidae	Y	N	N	N	Y	N	N	N	Y	N	Costa et al. (2021)
<i>Bombus impatiens</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Crall et al. (2017)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Dosselli et al. (2016)
<i>Bombus terrestris</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Evans et al. (2017)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Evans et al. (2019)
<i>Bombus terrestris</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Feltham et al. (2014)
<i>Bombus terrestris</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Gill & Raine. (2014)
<i>Bombus terrestris</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Hall et al. (2021)
<i>Rhynchophorus ferrugineus</i>	Dryopithoridae	Y	N	N	N	N	N	N	N	Y	Y	Hamidi et al. (2017)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	Y	N	N	N	N	N	He et al. (2013)
<i>Apis cerana</i>	Apidae	Y	Y	N	N	Y	N	N	N	Y	N	He et al. (2013)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	He et al. (2016)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Jingliang et al. (2020)
<i>Apis mellifera</i>	Apidae	Y	N	N	N	Y	N	N	N	N	N	Lach et al. (2015)
<i>Pollistes canadensis</i>	Vespidae	Y	N	Y	N	N	N	N	N	N	N	Lengronne et al. (2021)
<i>Apis mellifera</i>	Apidae	Y	N	N	N	Y	N	N	N	N	N	Li et al. (2013)
<i>Bombus terrestris</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Maurer et al. (2020)
<i>Bombus impatiens</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Minahan et al. (2018)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Minahan et al. (2018)
<i>Melipona fasciculata</i>	Apidae	Y	N	N	N	Y	N	N	Y	N	N	Nunes-Silva et al. (2020)
<i>Apis mellifera</i>	Apidae	Y	N	N	N	Y	N	N	N	N	N	Okubo et al. (2020)
<i>Bombus impatiens</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Orbán et al. (2014)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Perry et al. (2015)
<i>Vespa velutina</i>	Vespidae	Y	Y	N	N	Y	N	N	N	N	N	Poidatz et al. (2018)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Prado et al. (2020)
<i>Bombus impatiens</i>	Apidae	Y	Y	N	N	N	N	N	N	N	N	Russell et al. (2017)
<i>Bombus terrestris</i>	Apidae	Y	Y	N	N	Y	N	N	N	N	N	Stanley et al. (2016)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	N	N	N	N	N	Y	Tenczar et al. (2014)
<i>Apis mellifera</i>	Apidae	Y	Y	N	N	Y	N	N	N	Y	N	Thompson et al. (2016)
<i>Melipona quadrifasciata</i>	Apidae	Y	N	N	N	N	N	N	N	Y	N	Toppa et al. (2021)
<i>Apis mellifera</i>	Apidae	Y	N	N	N	N	N	N	N	N	Y	Van Geystelen et al. (2016)

**Table 1.3.3:** Summary of case studies using Harmonic radar (N=31). (N=36 when papers with more than one study species are accounted for).

Insect characteristics			Study aims										Maximum range (m)	Case study
Species	Family	Flying	FOR	MOV	HAB	IMP	NAV	EFF	SOC	DIS	MIG			
Apis mellifera	Apidae	Y	N	N	N	Y	N	N	N	N	N	600	Beyaert <i>et al.</i> (2012)	
Bombus terrestris	Apidae	Y	N	N	N	Y	N	N	N	N	N	1000	Brebner <i>et al.</i> (2011)	
Apis mellifera	Apidae	Y	N	N	N	Y	N	N	Y	N	N	270	Degen <i>et al.</i> (2015)	
Apis mellifera	Apidae	Y	N	N	N	Y	N	N	Y	N	N	1000	Degen <i>et al.</i> (2018)	
Apis mellifera	Apidae	Y	N	N	N	Y	N	N	Y	N	N	1000	Fischer <i>et al.</i> (2014)	
Apis mellifera	Apidae	Y	N	N	N	Y	N	N	Y	N	N	900	Greggers <i>et al.</i> (2013)	
Bactrocera minax	Tephritidae	Y	N	N	Y	N	N	Y	Y	N	N	30	He <i>et al.</i> (2019)	
Lycorma delicatula	Fulgoroidea	N	N	N	N	N	N	N	N	Y	Y	10	Jung <i>et al.</i> (2016)	
Riptortus pedestris	Alydidae	Y	N	N	N	N	N	N	N	N	Y	600	Kho <i>et al.</i> (2019)	
Lycorma delicatula	Fulgoroidea	Y	N	N	N	N	N	N	N	Y	N	30	Kim <i>et al.</i> (2016)	
Ricania speculum	Ricaniidae	Y	N	N	N	N	N	N	N	Y	N	30	Kim <i>et al.</i> (2016)	
Apis mellifera	Apidae	Y	N	N	N	N	N	N	N	Y	N	30	Kim <i>et al.</i> (2016)	
Bombus terrestris	Apidae	Y	N	N	N	N	N	N	N	Y	N	30	Kim <i>et al.</i> (2016)	
Riptortus pedestris	Alydidae	Y	N	N	N	N	N	N	N	Y	N	30	Kim <i>et al.</i> (2016)	
Halyomorpha halys	Pentatomidae	Y	N	N	N	N	N	N	N	Y	Y	50	Kirkpatrick <i>et al.</i> (2019)	
Halyomorpha halys	Pentatomidae	Y	N	N	N	N	N	N	N	Y	Y	200	Lee <i>et al.</i> (2013)	
Halyomorpha halys	Pentatomidae	Y	N	N	N	N	N	N	N	Y	Y	30-50	Lee <i>et al.</i> (2014)	
Riptortus pedestris	Alydidae	Y	N	N	N	N	N	N	N	Y	N	30	Lee (2016)	
Leucothrips caudalis	Anisoptera	Y	N	N	N	N	N	Y	N	Y	N	120	LeNaour <i>et al.</i> (2019)	
Vespa velutina	Vespidae	Y	N	N	N	N	N	N	Y	N	Y	500	Lioy <i>et al.</i> (2021)	
Vespa velutina	Vespidae	Y	N	N	N	N	N	N	N	N	Y	470	Maggiore <i>et al.</i> (2019)	
Bombus terrestris	Apidae	Y	N	N	Y	N	N	N	N	Y	N	800	Makinson <i>et al.</i> (2019)	
Carabus granulatus	Carabidae	Y	N	N	Y	N	N	N	Y	N	N	2.00	Martay <i>et al.</i> (2014)	
Vespa velutina	Vespidae	Y	N	N	N	N	N	N	N	N	Y	125	Milanesio <i>et al.</i> (2016)	
Vespa velutina	Vespidae	Y	Y	N	N	N	N	N	N	N	Y	150	Milanesio <i>et al.</i> (2017)	
Halyomorpha halys	Pentatomidae	Y	N	N	N	N	N	Y	Y	N	Y	30-50	Morrison <i>et al.</i> (2016)	
Bombus terrestris	Apidae	Y	Y	N	N	N	N	N	N	N	N	700	Osborne <i>et al.</i> (2013)	
Bombus terrestris	Apidae	Y	Y	N	N	N	N	N	N	N	N	700	Pasquaretta <i>et al.</i> (2021)	
Nezaraviridula	Pentatomidae	Y	N	N	N	N	N	Y	Y	N	N	5	Pillay <i>et al.</i> (2013)	
Apis mellifera	Apidae	Y	N	N	N	Y	N	N	N	N	N	900	Sol Balbuena <i>et al.</i> (2015)	
Apis mellifera	Apidae	Y	N	N	N	Y	N	N	N	N	N	600	Wolf <i>et al.</i> (2014)	
Apis mellifera	Apidae	Y	N	N	N	Y	N	N	N	N	N	600	Wolf <i>et al.</i> (2016)	
Bombus terrestris	Apidae	Y	Y	N	N	N	N	Y	Y	N	N	600	Woodgate <i>et al.</i> (2016)	
Bombus terrestris	Apidae	Y	Y	N	N	Y	N	N	Y	N	N	600	Woodgate <i>et al.</i> (2017)	
Apis mellifera	Apidae	Y	N	N	N	Y	N	Y	N	N	N	600	Woodgate <i>et al.</i> (2021)	
Monochamus alternatus	Cerambycidae	Y	N	N	N	N	N	N	N	Y	Y	150	Zhang <i>et al.</i> (2020)	

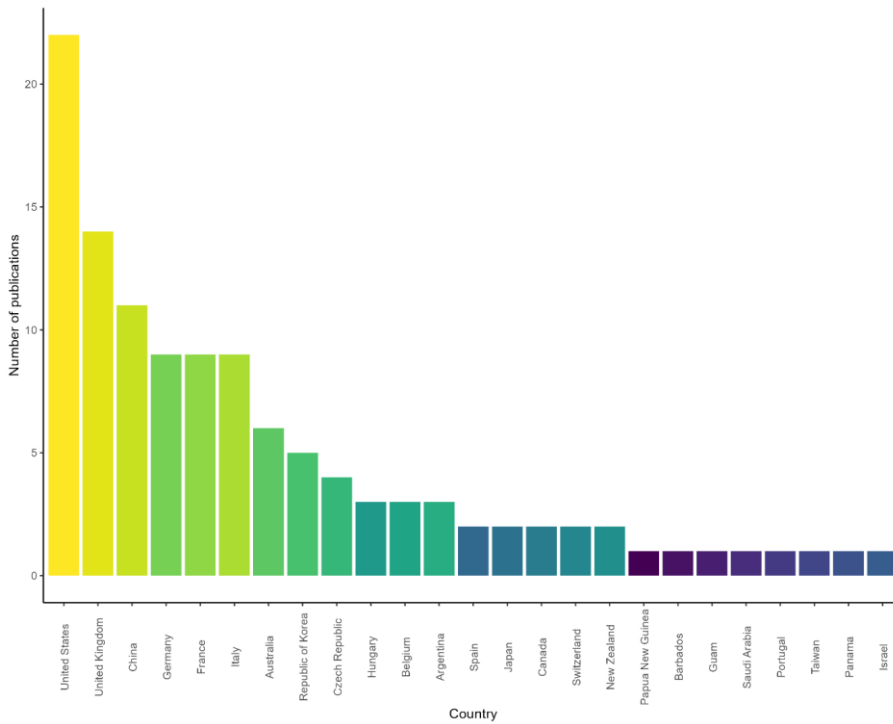
**Table 1.3.4:** Summary of case studies using 2-dimensional barcodes (N=11). (N=18 when papers with more than one study species are accounted for).

Insect characteristics			Study aims										Case study
Species	Family	Flying	FOR	MOV	HAB	IMP	NAV	EFF	SOC	DIS	MIG		
Apis mellifera	Apidae	Y	Y	N	N	N	N	N	N	N	N	Barascou <i>et al.</i> (2022)	
Apis mellifera	Apidae	Y	N	Y	N	N	N	N	N	N	N	Blut <i>et al.</i> (2017)	
Apis mellifera	Apidae	Y	N	N	N	Y	N	N	N	N	N	Bordier <i>et al.</i> (2017)	
Bombus impatiens	Apidae	Y	N	N	N	N	N	N	N	N	Y	Du <i>et al.</i> (2022)	
Apis mellifera	Apidae	Y	N	Y	N	N	N	N	N	N	N	Germat <i>et al.</i> (2018)	
Camponotus fellah	Formicidae	N	N	Y	N	N	N	N	N	N	N	Heyman <i>et al.</i> (2017)	
Apis mellifera	Apidae	Y	Y	N	N	N	N	N	N	N	N	Jones <i>et al.</i> (2020)	
Lasius niger	Formicidae	N	N	Y	N	N	N	N	N	N	N	Planckaert <i>et al.</i> (2019)	
Apis mellifera	Apidae	Y	Y	N	N	N	N	N	N	N	N	Prado <i>et al.</i> (2020)	
Lasius niger	Formicidae	Y	N	Y	N	N	N	N	N	N	N	Richardson <i>et al.</i> (2022)	
Termitothorax nylanderi	Formicidae	Y	N	Y	N	N	N	N	N	N	N	Richardson <i>et al.</i> (2022)	
Leptothorax acervorum	Formicidae	Y	N	Y	N	N	N	N	N	N	N	Richardson <i>et al.</i> (2022)	
Apis mellifera	Apidae	Y	N	Y	N	N	N	N	N	N	N	Richardson <i>et al.</i> (2022)	
Apis mellifera	Apidae	Y	Y	Y	N	Y	N	N	N	N	N	Smith <i>et al.</i> (2022)	
Bombus impatiens	Apidae	Y	Y	N	N	N	N	N	N	N	Y	Smith <i>et al.</i> (2022)	
Bombus bimaculatus	Apidae	Y	Y	N	N	N	N	N	N	N	Y	Smith <i>et al.</i> (2022)	
Bombus griseocollis	Apidae	Y	Y	N	N	N	N	N	N	N	Y	Smith <i>et al.</i> (2022)	
Bombus perplexus	Apidae	Y	Y	N	N	N	N	N	N	N	Y	Smith <i>et al.</i> (2022)	



### 1.3.1. General characteristics of publications

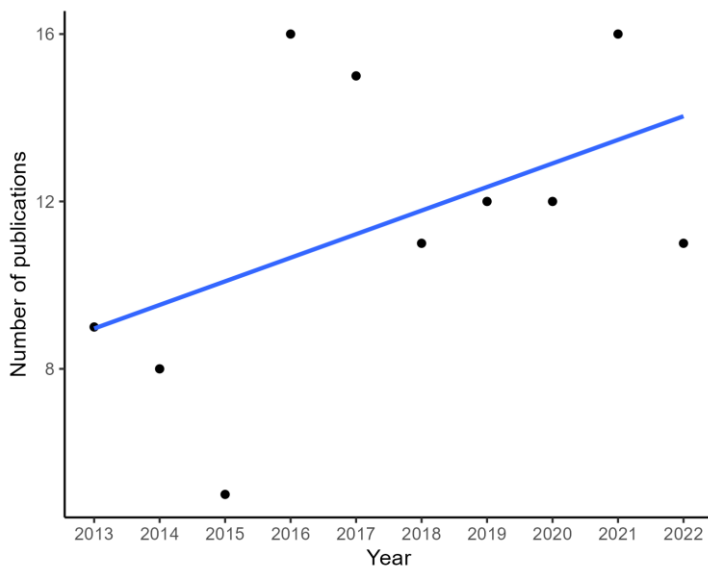
Figures 1.3.1, 1.3.2, and 1.3.3 categorise the 116 publications included in this systematic map by country of study, year of publication, and insect family studied, respectively.



**Figure 1.3.1:** Number of publications by country of study.

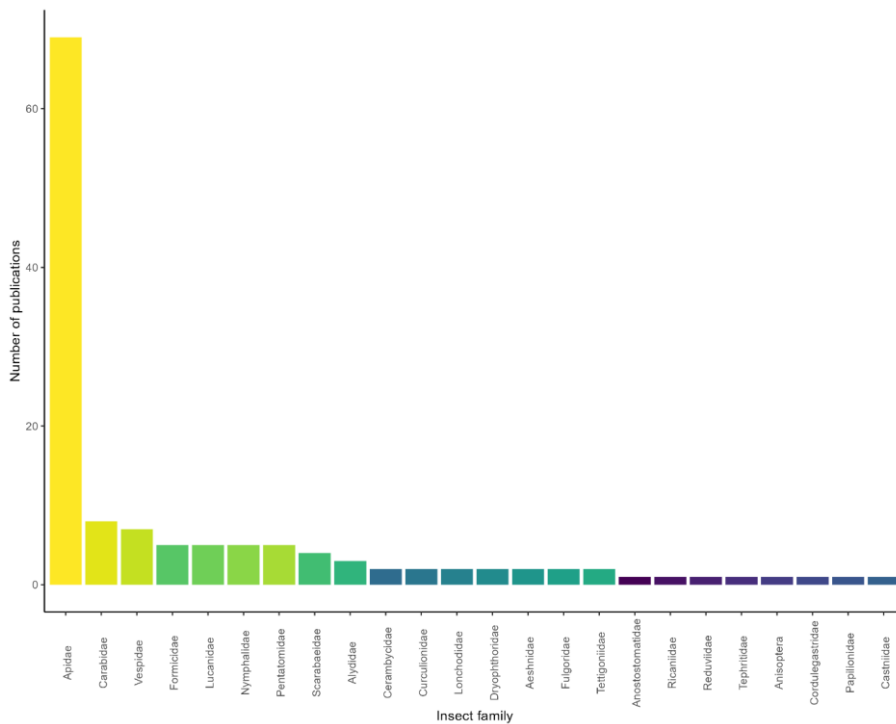
The majority of studies were conducted in Europe ( $n=56$ ), with 73% of those studies conducted across four countries (UK, 14; Germany, 9; Italy, 9; France, 9) (figure. 1.3.1). Multiple studies were also conducted in North America ( $n=24$ ) (USA, 22; Canada, 2), and East Asia ( $n=13$ ) (China, 11; Republic of Korea, 5; Japan, 2; Taiwan, 1). Other areas where fewer studies were conducted were Oceania ( $n=9$ ) (Australia, 6; New Zealand, 2; Papua New Guinea, 1) and South America ( $n=3$ ) (Argentina, 3).

When creating Figure 1.3.2, I excluded the year 2012 because it did not represent a complete year in my literature search. The maximum number of papers published in a single year was 16 in 2016, and 2021, with 28% of all publications included in the review published during these years. The minimum number of papers published in a single year was five in 2015, constituting 4% of all publications included in the review.



**Figure 1.3.2:** Number of publications from each year from 2013 to 2022.

The most commonly studied insect family was Apidae (bees) (52%) (figure 1.3.3). The second most commonly studied insect family was Carabidae (beetles), constituting 6% of studies in my review. The remaining 21 insect families studied in the review each constitute 5% or less, with the majority of families appearing in two or fewer studies.



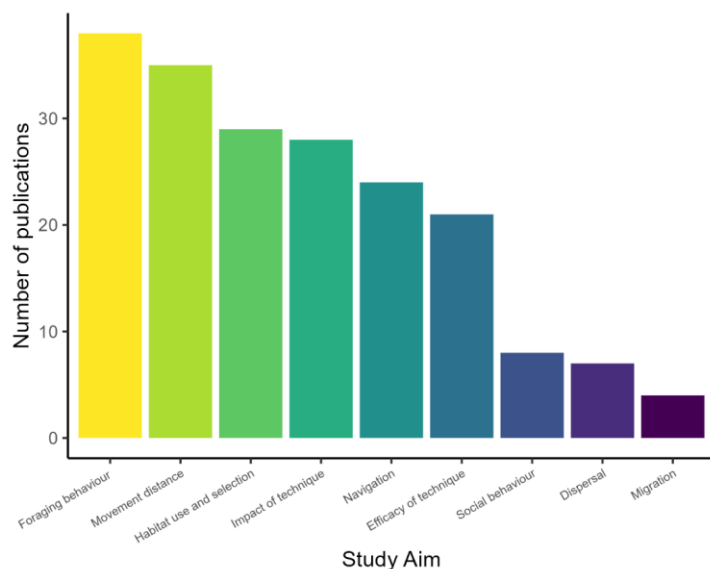
**Figure 1.3.3:** Number of publications versus insect family. A single study can count multiple times if it contains more than one studied insect family.

### 1.3.2. Telemetry technology and study aims

Figure 1.3.4 categorises the 116 publications included in this systematic map by study aim.

The study aims of the publications included in this review can be grouped into nine categories: foraging behaviour, movement distance, habitat use and selection, impact of technology, navigation, efficacy of technology, social behaviour, dispersal and migration. Categorisation criteria are included in supplementary information 1.1.

Forty-seven percent of publications in my review addressed more than one study aim. The most commonly addressed study aim was foraging behaviour (33%), followed by movement distance (30%). Only 3% of publications investigated migration.



**Figure 1.3.4:** Number of publications versus main study aims. The main study aims are categorised as foraging behaviour, movement distance, habitat use and selection, impact of the telemetry technology, navigation, efficacy of the telemetry technology, social behaviour, dispersal, and migration. A single study can count more than once.

RFID was the most commonly used telemetry technology (34%), followed by radio telemetry (30%), harmonic radar (28%), and 2D barcode tags (10%). The majority of the publications in my review investigated flying insect species (87%).

## 1.4. Discussion

### 1.4.1. Comparison to Kissling *et al.*, 2014

In 2014, Kissling *et al.* published a review of the history of insect telemetry, summarising the progression of the field since its inception in 1954 (85). Kissling *et al.* reviewed literature on insect telemetry using radio telemetry, and compared the technology to harmonic radar and RFID. Because my literature search collates publications on insect telemetry using radio telemetry, harmonic radar, RFID, and 2D barcodes, I cannot directly compare this data set

with those of the Kissling *et al.* review. However, the Kissling *et al.* review is the most complete review of insect telemetry prior to 2012, and provides a starting point to explore how the field of insect telemetry has developed in the last decade.

#### 1.4.2. Discussion on general characteristics

My data show an upward trend in insect telemetry research published between 2013 and 2022. Although this trend mirrors previous reviews on insect telemetry, as well as general trends in entomological science publication rates (85, 127), it may also reflect technological advancements making insect telemetry more accessible to researchers in recent years.

Insect telemetry is a developing field, and this is reflected by the significant rise in research output over time. Kissling *et al.* (2014) collected 27 publications on insect telemetry using radio telemetry published prior to October 2012. Meanwhile, my review identified 35 publications in this category between October 2012 and February 2023, showing a marked increase in output of research using radio telemetry on insects over the last decade. Moving forwards, I expect this trend to continue as technological advancements make insect telemetry a cheaper, more effective, and therefore more attractive research technique.

Eighty-seven percent of the publications in this review used insect telemetry to study flying insect species. Studies on insect species from the Apidae (bees) family are overrepresented, constituting 60% of publications on flying insect species, and 52% of all publications included in the review.

The biased usage of RFID partly explains the overrepresentation of Apidae. Due to its very short detection range and its uniquely coded tags, RFID allows researchers to monitor entire colonies using a reader positioned at the entrance to the nest. Thus, it is highly suitable for studying social insect families such as Apidae and Vespidae. Indeed, 90% of publications using RFID studied Apidae, and of those, 63% studied *Apis* sp. (honeybees) (Table 1.3.2).

Fifty percent of publications using harmonic radar studied insect species from the Apidae family, further highlighting the overrepresentation of bees in this review. However, if publications using RFID and harmonic radar are discounted, a different trend emerges. Sixty-nine percent of studies on non-flying insect species used radio telemetry, reflecting the limitations of RFID and harmonic radar when studying non-flying insect species. However, in

my review, the majority of publications using radio telemetry (69%) studied flying insect species (Table 1.3.1). This is more than the Kissling *et al.* (2014) review in which 48% of publications studied flying insect species, and suggests radio telemetry is becoming more suitable for use on flying insect species. Despite this, in both reviews, insect species from the Apidae family represented a small proportion of publications using radio telemetry. As previously discussed, radio telemetry is, on average, the heaviest insect telemetry technology, making it less suitable for use on smaller, lighter insect species (128). Although the increase in studies on flying insect species suggests that tag mass may have reduced, I suggest that further technological development is needed to improve the viability of radio telemetry for use on insects.

I show the majority of insect telemetry studies are concerned with larger, more robust species such as bees (figure 1.3.3). To ensure future studies are capable of tracking a wider variety of species and, indeed, smaller species, telemetric technology requires miniaturisation. Technology is the limiting factor for study species and research questions asked. Currently, questions surrounding Apidae are more frequent than other insect families, resulting in the biases seen here. This bias is partly explained by the fact that insect tracking technologies were pioneered with bees (95, 118). Most tags can be attached and carried by bees easily but these same protocols are not easily transferrable across species e.g. in slugs RFID tags required surgical implantation (129). In order to address species bias, current and emerging technologies should be lighter and smaller. These improvements would allow the tags to be attached and carried by a much wider range of insect species and reduce the bias we see towards Apidae.

The majority of studies in my review were conducted in Europe (n=56) and North America (n=24), constituting 69% of all publications. It is important to note that I conducted the literature search in English, and therefore, only papers published in English were included. This highlights a limitation of my literature search that may have led to some publications being excluded from the review. However, the geographical trends that we see here are consistent with those of the International Scientific Journal & Country Ranking portal when ranking Insect Science journals by research output within the last three years (127). These geographical trends persist when looking at the most popular insect family in my review,

Apidae. sixty-seven percent of Apidae papers were published in Europe (n=31) and North America (n=10), and 61% of all honeybee papers were published in Europe (n=19) and North America (n=4).

#### 1.4.3. Discussion on telemetry technology and study aims

RFID was the most commonly used tracking technology (34%), followed by radio telemetry (30%), harmonic radar (28%) and 2-dimensional barcodes (11%). The harmonic radar is the most expensive telemetry technology used in our review when using a scanning parabolic radar system (130). However, the use of alternative harmonic radar technologies is increasing in popularity, as well as studies focussing only on the impact of tag attachment. Overall, 16 (50%) of the 32 publications using harmonic radar used a scanning parabolic radar dish. Meanwhile, seven (22%) used a handheld version, 5 (16%) used a portable lightweight version (<10kg), and 4 (13%) studied the impact of tag attachment without using a receiver. Moreover, the trends in the types of harmonic radar use reflect not only their cost-effectiveness but also the adaptability and versatility of these technologies in ecological research. As more studies focus on the specific impact of tag attachment, we see a shift towards more lightweight and portable versions of harmonic radar. These alternatives not only offer cost savings but also provide researchers with the flexibility to conduct fieldwork in diverse environments. It is noteworthy that these developments align with the broader goal of minimizing the impact on the study species and preserving the integrity of research findings. As such, I predict the parabolic harmonic radar will become increasingly less popular in the future due to these new, cheaper, and more accessible alternatives.

Foraging behaviour was the most common study aim in the review (Fig. 1.3.4). It is important to note that insect telemetry studies cannot directly measure behaviour. Insect telemetry quantifies movement, and researchers interpret the data for their study aim. For example, when using RFID to track honeybees leaving and exiting the hive when infected with a parasite, Dosselli *et al.* were able to interpret the data as foraging behaviour (131). Furthermore, study aims influence technology and vice versa, as demonstrated by the fact that 72% of publications using RFID studied foraging behaviour non-exclusively (Table 1.3.2). RFID has a limited detection range, as previously discussed, and this limits its uses. However,

it can be used to track movement patterns if readers are set up in different locations – for example at a nest entrance and feeding station (80).

Movement distance was the second most common study aim in my review (30%), followed by habitat use and selection (25%). Only four (3%) publications studied migration, and all used radio telemetry. Tag mass and therefore detection range are the key limiting factors when studying migration in the field of insect telemetry. The active tags used in radio telemetry are too small to support enough power to track insects at long range (>1km), and the range of the passive tags used in RFID and harmonic radar is even more limited.

The migration studies in our review circumvent these limitations either by studying non-flying, relatively large (3400-4100mg) insect species that migrate across easily traversable terrain (132) or by covering the entire migratory path with telemetry towers. In this second example, Knight *et al* (133) used an automated array of over 100 telemetry towers across Southern Ontario, Canada and the north of the United States to monitor the autumn migratory patterns of monarch butterflies (*Danaus plexippus*) and green garner dragonflies (*Anax junius*). They found that monarch butterflies travelled an average of 61km per day with a maximum daily flight distance of 143km, and green darner dragonflies travelled an average of 43km a day with a maximum daily flight distance of 122km. This study represents, to our knowledge, the furthest tracking distance of any insect telemetry paper achieved to date. However, the methods used in this publication are unrealistic for most researchers, as they rely on pre-existing infrastructure and/or significant investment of resources. Furthermore, Knight *et al* (133) acknowledge potential limitations of their methodology, noting that both the impact of tag mass on the insects as well as the limited detection range and functionality of the telemetry towers may have influenced their results.

Building on the capabilities of insect telemetry to accurately quantify migration patterns, this technique also proves invaluable in shedding light on the dynamics of insect dispersal. Here, dispersal is defined as the movement of an individual between its natal site and the potential location of reproduction (134). It was the second least common study aim in our review, behind migration. In many insect species, dispersal occurs across the landscape scale (>1km) (52), and, as such, it would require a tracking technology with high durability and detection range in order to quantify.



Our literature search revealed that dispersal studies primarily utilize radio telemetry or harmonic radar, both known for their comparatively long detection ranges (Table 1.3.1 and 1.3.3). Hence, the relative rarity of dispersal studies is likely due to the extensive time and distance required for tracking. Consequently, the preference for radio telemetry and harmonic radar in such studies aligns with their extended detection range and durability, as demonstrated in our systematic map.

It may be possible to address these biases by combining free-flying tracking techniques with tethered methods. In their review of tracking methods in bumblebees, Mola and Williams (135) suggest incorporating tethered flight mills to mitigate biases in tracking techniques. By introducing flight mills, researchers can quantify movement capacity (136). Additionally, they conclude that this complementary approach would eliminate the influence of cognitive factors affecting movement, allowing research to focus on the physical capacity under controlled scenarios. The same principle can be extended to all insect species in cases where technological biases or constraints exist, such as dispersal in our review.

While radio telemetry and harmonic radar, with their high detection ranges, are crucial in understanding insect dispersal, all four tracking techniques reported in this systematic map (RFID, radio telemetry, harmonic radar, and 2D barcodes) explore navigation as a study aim, regardless of their detection ranges. Navigation was a main study aim in twenty-four papers (21%), and among them, forty-six percent utilized RFID, while an additional forty-six percent employed harmonic radar. Researchers are able to use each of these different tracking techniques to investigate the same study aim, highlighting the versatility of these techniques. When interested in insect navigation, researchers are able to choose the technique that best suits their specific needs and constraints, making their study adaptable to different research scenarios. For example, the abilities of species to return home when stressed can be quantified by both harmonic radar (39) and RFID (80). Each of these papers is able to use the selected tracking technique to its advantage, likely because the technique's efficacy had been taken into account during the experimental design process.

Twenty-one (18%) publications studied the efficacy of telemetry technology, researching not only the suitability of the technology but also testing improvements. For example, Fisher *et al.* (137) devised the first automated radio telemetry system designed for non-migratory

insect telemetry. Capable of estimating locations of butterflies equipped with low-power tags every five seconds, the technology was designed to overcome many of the limitations of existing insect telemetry technologies. The authors state that their findings likely represent the limit of existing automated radio telemetry technologies for non-migratory movement of insects. However, they acknowledge their large error estimates, highlighting the difficulty of developing new insect telemetry technologies.

Twenty-eight papers (24%) studied the impact of telemetry technology on the behaviour of tagged insects. In telemetry studies, the fundamental objective is to obtain precise and reliable data concerning an insect's movement and behaviour. However, tag attachment is a potential factor influencing insect behaviour, potentially leading to altered behaviours or hindrances in the accurate recording of tracking data. Therefore, in the pursuit of successful insect telemetry studies, it is imperative for researchers to strike a balance between optimizing tracking accuracy and minimizing disruptions to the insect's natural behaviour. Consequently, the quantification of any potential impacts of the technology should be a preliminary requirement. Nevertheless, it is worth noting that a substantial portion of the publications reviewed in our study expressed concerns regarding the impact of telemetry technology on insect behaviour without quantifying these effects, as exemplified by Knight *et al.* As a result, the available data on the impact of telemetry technology may be incomplete.

Twenty-three of the 35 (66%) publications using radio telemetry studied movement distance. Radio telemetry's long detection range and manoeuvrability make it an attractive technology to researchers studying movement distance across difficult landscapes (e.g. Wang *et al* used high frequency radio telemetry to track multiple Golden Birdwing butterflies [*Troides aeacus*] simultaneously across a mountainous habitat (138)). A total of 29 papers studied habitat use and selection, and all of these used radio telemetry (83%) or harmonic radar (17%), reflecting the significant limitations of 2D barcodes's and RFID's detection ranges.

As previously mentioned, RFID was utilized in a high number of papers on foraging behaviour and many (55%) of these focussed on honeybees. RFID technology has allowed for the high-throughput, real-time quantification of honeybee colony foraging behaviour. For example, RFID was used to quantify the deleterious impacts of both disease (100, 102, 131, 139) and

field realistic doses of the neonicotinoid pesticide, Thiamethoxam, on honeybee foraging and survival (140).

Social behavior was a study aim in forty-six percent of 2D barcode studies. The protocol for using most 2D barcode systems is well positioned to quantify the in-nest social interactions of insect species such as social bees and ants (108, 109, 141). 2-dimensional barcodes enable the collection of such data in situations where other techniques are unsuitable. Each tracking technique has its strengths, and, as a result, we would expect that certain study objectives may be predominantly investigated using specific tracking techniques. This is evident in the case of social behavior and 2D barcodes in insect telemetry.

Indeed, all the studies in this review that utilized 2D barcodes focused on species from the Apidae and Formicidae families (see Table 1.3.4). By tagging entire colonies and tracking the in-nest movements of multiple individuals simultaneously, researchers can observe and quantify the utilization of space and social structure within the colonies. Social insect nests contain specific boundaries and chambers, which affect individual-level interactions, resource allocation, and division of labor (142). The use of two-dimensional barcodes have revealed that three species of ants and one species of honeybee adjust their movement patterns depending on the 'task zone' within their nest (141). Researchers have also discovered that all these species exhibit boundary effect mechanisms, wherein physical boundaries and structures within the nest influence their behaviour (141).

### 1.5. Future prospects in insect telemetry

This systematic map shows that the field of insect telemetry has developed considerably since the Kissling *et al.* (2014) review. The volume of insect telemetry research is increasing, and technological developments improve the scope of insect telemetry all the time. However, the trends identified in my discussion expose a number of areas where research is currently lacking. Kissling *et al.* identified many of these problems more than a decade ago, highlighting the continued need for further developments in the field of insect telemetry. I discuss these much-needed developments in detail below.

### 1.5.1. Improved diversity across insect telemetry

Kissling *et al.* (2014) recommended that more insect telemetry research should be conducted in tropical regions, given that most insect species are predicted to occur at tropical latitudes (143). However, tropical species remain underrepresented in my review, highlighting a continued need for insect telemetry research on tropical species.

Improvement in this area would also alleviate the need to study a broader range of species. Indeed, this review shows that species from the Apidae family are significantly overrepresented in insect telemetry research. In order to ensure policies and agri-environment schemes are well rounded, research on lesser-studied insect species should be prioritised. However, even within the Apidae family, more diversity is needed. The majority of Apidae studies focus on honeybee workers and, to a lesser extent, bumblebee workers. These species represent a fraction of Apidae species worldwide, and therefore, more research is needed on other lesser understood species and castes that may be more vulnerable to environmental stressors (63, 144, 145).

Furthermore, research on Apidae is heavily biased towards western countries, and this does not reflect the global distribution of this insect family. Although *A. mellifera*, for example, are present throughout most of the world, most insect telemetry data on this species has been collected in a handful of countries. Future research should aim to use insect telemetry on species from Apidae in a wider range of countries outside of Europe and North America. Every country has its own unique landscapes and environmental differences, which may influence the behaviour of the organisms living within them, and therefore how they respond to stressors (e.g. agrochemicals, pathogens, dietary stresses). A study of the impacts of a pesticide on *A. mellifera* in the UK, for example, should not be extrapolated to cover all uses of the same pesticide on *A. mellifera* globally. Furthermore, the geographical trend towards western countries seen in studies on Apidae reflects the general geographical trends seen in my review. This highlights the need for more geographical diversity across insect telemetry.

Finally, many of the publications in this review focus on a single caste within a species (e.g. honeybee foragers (39, 140, 146)). Future studies should attempt to quantify intraspecific

variability between castes and individuals in order to create a complete picture of individual species and their interactions with their species-specific environment.

#### 1.5.2. Impact of technology

Kissling *et al.* (2014) (85) raised the importance of quantifying the impact of telemetry technologies on insect behaviour. However, my review highlights a lack of data in this area, and further emphasises the pressing need to quantify impact of existing telemetry technologies across the field.

Furthermore, as technology continues to advance, new methods will become available to researchers (89, 147). Understanding a technique's impact on normal organismal behaviour should be a prerequisite for telemetry experiments. Thus, the first studies using novel telemetry technologies (especially on novel target species and castes) should aim to quantify the potential impact of the telemetry technology on the behaviour of the individual, as well as its efficacy as summarised by Batsleer *et al.* (84). Researchers must strike a balance between the benefits of telemetry technologies and their potential side effects to ensure accurate data.

#### 1.5.3. Further technological advancements

My review highlights the difficulty of developing telemetric technologies for use on insects. Although technological advancements have been made since the Kissling *et al.* (2014) review, further development is needed, especially with regards to detection range and tag mass.

To identify and preserve key species-specific resources, it is critical that we understand how insects use their environment at a landscape scale (>1km). Insect telemetry data at this scale may prove invaluable for conservation efforts and ultimately aid in addressing insect species declines (148). However, my review demonstrates that landscape scale data are lacking across the field of insect telemetry, and this is because of the limitations of technologies at present [this sentiment is shared in bumblebee movement ecology (135)].

Improvements to the detection range and tag mass across insect telemetry technologies are sorely needed. Furthermore, additional technological advancements (e.g. automated systems, further miniaturisation etc.) may improve the prospects of future insect telemetry

research. Kissling *et al.* (2014) suggest the use of satellite-based tracking systems to monitor migratory species, for example. However, this review shows that tracking of migratory species is possible without the need for such systems. Therefore, researchers should review any further development to existing technologies, as well as new technologies, in order to ensure that they are necessary for achieving the goals of the study.

## 1.6. Conclusion

This systematic map identified 116 papers published between October 2012 and February 2023 using insect telemetry. I found RFID to be the most frequently used tracking technology, foraging behaviour the most common study aim, and Apidae to be the most studied insect family. I also identified an upward trend in the number of papers assessing the impact of technology on normal insect behaviour.

The results of this systematic map uncover a number of shortcomings in the field of insect telemetry in its current state. As the field continues to grow, I recommend future studies quantify species, caste, and habitat specific impacts of tracking technologies on normal organismal behaviour with a view to further development of these technologies to allow for use on a broader range of species from different insect families.

## **Chapter 2**

The impact of sulfoxaflor and  
flupyradifurone on *Bombus terrestris*

# flight characteristics assessed by flight mill and video footage

## 2. Chapter 2: The impact of sulfoxaflor and flupyradifurone on *Bombus terrestris* flight characteristics assessed by flight mill and video footage

### 2.1 Introduction

Bees provide the essential ecosystem service of pollination (72). Moreover, they account for 62% of flowering crop visitations (69) and support 9.5% of global food production (9). Declines in the number, diversity, and range of bees internationally (149, 150) are of major concern. Bumblebees in particular, contribute a significant proportion of pollination services, both as wild and as commercially bred pollinators (150, 151).

The decline of bee populations has been attributed to several key stressors including habitat loss and fragmentation, invasive species, climate change, pathogenic and parasitic infections, diminished food resources and pesticides (25, 30, 152, 153). In modern agricultural systems, bees are exposed to a diverse range of pesticides (34, 46, 154), often in combination (155-157). Thus, combined with the long foraging season of bumblebees (158, 159), there are numerous opportunities for combination exposure to agrochemicals.

In eusocial species, like bumblebees, a forager's ability to collect nectar and pollen for the colony relies on its ability to fly unhindered and frequently. Bumblebees have been recorded



flying up to 2.2km from their nest to forage (160-162) and perform multiple foraging bouts per day per bee (163, 164). An impairment of flight capabilities, such as endurance (165), and therefore foraging ability can detrimentally impact colony fitness (104).

Sulfoxaflor and flupyradifurone are the recently developed sulfoximine and butanolide-based insecticides, respectively, which have been registered for agricultural use (166, 167). Both share their mode of action with neonicotinoid pesticides, targeting nicotinic acetylcholine receptors (NACHRs), but differ in their chemical structure (166-170). They are applied as either a spray or seed treatment (166, 167). Such systemic insecticides are present in all tissues of the treated plant, including nectar and pollen. Beneficial insects, such as bumblebees, are either directly exposed during the spraying process; through contact exposure to dust sloughed off the treated seeds as they are planted; through contact with treated soils (e.g. hibernating bumblebee queens and solitary bees during their lifecycle (55, 171, 172)) or indirectly exposed when feeding on nectar and pollen.

More studies on the combined impacts of agrochemicals are urgently required (33). I aimed to quantify the impact two novel insecticides have on a key pollinator species' flight characteristics and foraging frequency. In this study, I assess the impact of chronic sulfoxaflor exposure on the flight abilities of *Bombus terrestris audax* workers using a rotational tethered flight mill apparatus. I also assess the impact of chronic exposure to flupyradifurone and sulfoxaflor, alone and in combination, on colony level foraging activity in an indoor flight arena. I hypothesise that chronic exposure to sulfoxaflor and chronic exposure to sulfoxaflor and flupyradifurone, in a fully crossed design, will affect the flight capabilities of bumblebee workers. While each of these two stressors is not believed to have a significant impact on the ability to fly or forage on their own (94), it is unclear whether they might interact in ways that lead to additive or synergistic effects. Even if the underlying mechanism behind such an interaction is not yet fully understood, it is important to consider the potential impacts of their combination.

## 2.2 Methods

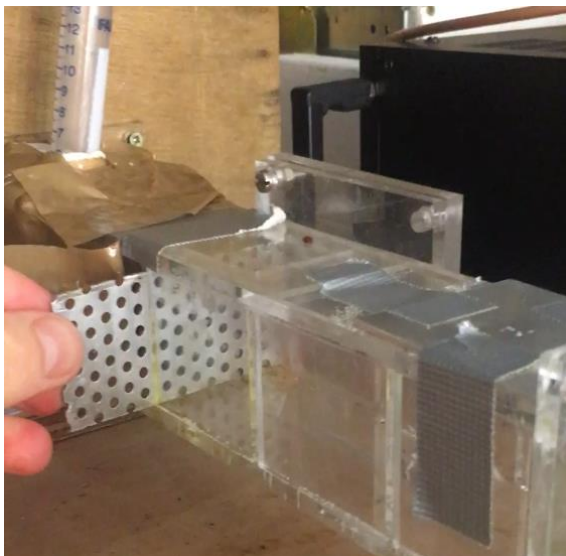
### 2.2.1. Flight mill bumblebee colonies

Ten *Bombus terrestris* colonies (*Bombus terrestris audax* research hives containing 40-50 workers) were purchased from Biobest, Belgium and placed in the lab at Rothamsted Research, Harpenden on 14th May 2019 (figure 2.2.1). Colonies were kept in a red-lit room maintained at 20 °C and 40-50% relative humidity. Colonies were transferred from their plastic nest boxes into wooden nest boxes (210mm x 294 mm x 181mm) with red Perspex lids. The lids featured a 6-inch diameter opening with a closable flap, which allowed the colony to



**Figure 2.2.1:** Ten bumblebee colonies delivered from BioBest before being transferred into wooden nest boxes.

receive pollen. Colonies were fed honeybee collected pollen (Agralan growers) *ad libitum*. A transparent Perspex tunnel (260mm x 40mm x 4mm) allowed individuals to leave the nest and could be blocked with a metal divider when necessary (figure 2.2.2).



**Figure 2.2.2:** The Perspex tunnel with metal divider that allows for control of bumblebee movement into and out of the nest box.

### 2.2.2. Insecticide dosage

Sulfoxaflor-based insecticides are used on a variety of different crops and is administered as a spray or seed treatment (166). As a systemic insecticide, sulfoxaflor is expressed throughout the tissue of the treated crop, including the flower's pollen and nectar. Beneficial insects, such as bees, can be directly exposed during spraying, or indirectly via feeding on nectar or pollen. The levels of residual systemic insecticides can differ significantly between crop species (173, 174). I based the dosage on Environmental Protection Agency (EPA) data that show the residue levels of sulfoxaflor range between 5.41 and 46.97ppb in the nectar of sulfoxaflor-sprayed cotton over an 11-day period (175)(application rate: 0.045 pounds (0.020 kg) of active ingredient per acre, applied twice). My decision on dosage was also influenced by previous research on sulfoxaflor exposure in *Bombus terrestris*. When chronically exposed to 5ppb sulfoxaflor, bumblebee colonies have reduced reproductive success and a reduction in egg laying and larval production (176-178). In combination with another stressor, the parasite *Nosema bombi*, chronic exposure to a 5ppb sulfoxaflor solution results in high mortality of *Bombus terrestris* larvae (179).

Flupyradifurone is also a systemic insecticide and is used as either a spray or seed treatment (167). In the same was as sulfoxaflor, beneficial insects may be exposed to flupyradifurone in agricultural environments. To determine the exposure dosages, I used data from the EPA which indicated that honeybees that foraged on oilseed rape treated with seed and spray applications of flupyradifurone were exposed to as much as 4.3 ppm in the nectar they collected (180). The pesticide was also found in honey and nectar stored in bee combs for up to five months and in the nectar collected by foragers over a period of more than two weeks (180).

### 2.2.3. Flight mill sulfoxaflor exposure

Before exposure began, five colonies were randomly assigned to the control and treatment groups, respectively. A stock solution was made from 10mg of sulfoxaflor (Greyhound Chromatography and allied chemicals) dissolved in 10ml of acetone at a concentration of 1000ppm. The stock solution was diluted to 100ml with acetone to create a solution with a

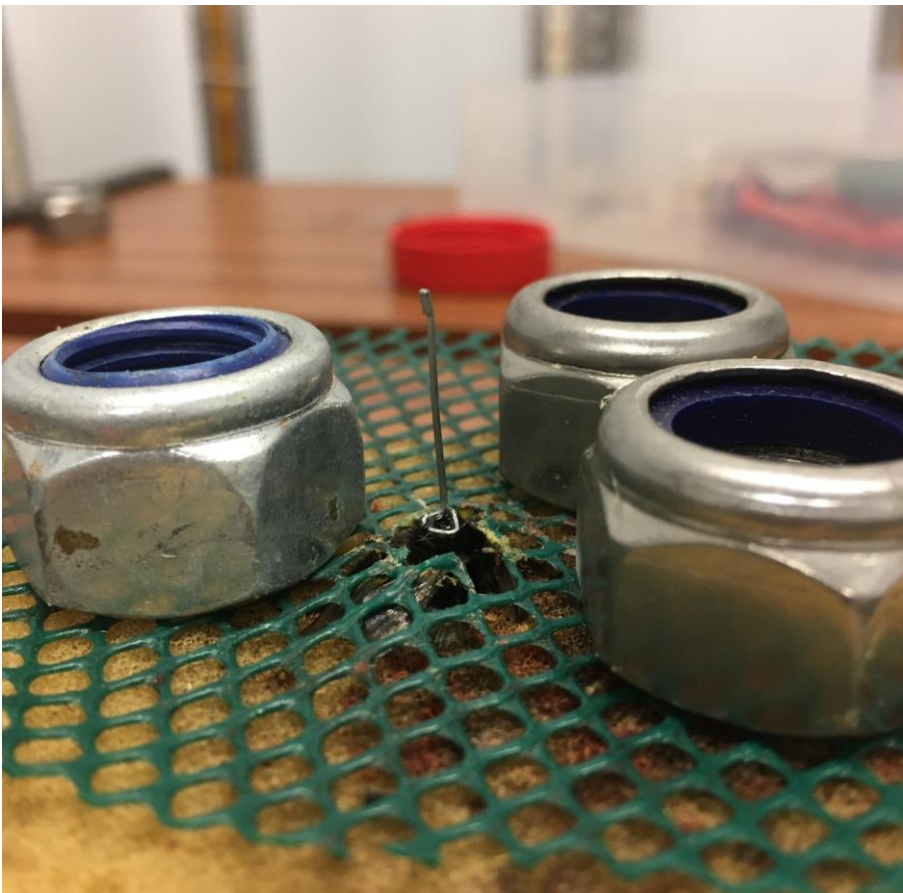
concentration of 100ppm. 50 $\mu$ l was removed from 1L of BioGluc<sup>®</sup> and 50 $\mu$ l of the 100ppm sulfoxaflor solution was then added to 1L of BioGluc<sup>®</sup> to create a 5ppb sulfoxaflor solution.

Five colonies were provided with an *ad libitum* supply of BioGluc<sup>®</sup> solution containing (5ppb) sulfoxaflor and five colonies were provided BioGluc<sup>®</sup> sucrose solution containing an equivalent concentration of acetone but no sulfoxaflor for 14 days. The colonies were able to access this solution by means of a 20ml Falcon tube with two 0.5mm holes drilled 2cm from the base. The Falcon tube was placed in the Perspex tunnel in 3cm dish to collect any excess solution. The quantity of solution consumed was recorded for both groups. There was no difference in the quantity of solution consumed between groups.

#### 2.2.4. Flight mill

Five bumblebee workers from each colony (control=25, sulfoxaflor=25) were attached to the flight mill apparatus. If the worker did not fly continuously for 20 seconds or more within the first ten minutes of attachment, it was removed from the mill. Attachment of workers was repeated until 50 workers (5 from each colony) had met this requirement. The age of workers nor the date of eclosure was recorded during the experiment.

Bumblebees were haphazardly selected and removed from the Perspex tunnel using forceps. Selected bumblebees were placed between plastic mesh and a sponge; held in place by three weights. A metal loop was shaped from 0.25mmx0.5mm steel wire and attached to the bumblebee using an adhesive (Gorilla Super Glue Gel) on the dorsal side of the thorax (Fig.



**Figure 2.2.3:** Preparing bumblebees for tethered flight, holding the bumblebee in place to allow the adhesive to successfully bond.

2.2.3).

The flight mill consists of a flight arm made from twisted wire with the vertical axis secured between two magnets. The loop, once attached to the bumblebee, is then fitted into a plastic sleeve suspended from the flight arm and this allows the bumblebee to fly rotationally in a

horizontal plane. Data are collected as an attached banded disc passes through a light sensor that records the speed and time of rotation. Because bumblebees rely mostly on visual cues for their flight, the flight mills were enclosed by a screen with uniform black-and-white stripes to provide optical flow for the flying bumblebees (see figure 2.2.4). The screen had several benefits, including the prevention of potential interfering air currents that can affect



**Figure 2.2.4:** The flight mill apparatus with bumblebee worker mid-flight. Screens surrounding the mill provide optical flow for the attached bumblebee.

bumblebee hesitancy during flight take-off (181); relative isolation from neighbouring flight mills; and presenting an identical visual stimulus that could be a contributing factor in determining flight speed (182).

Eight flight mills were used simultaneously. While attached to the flight mill, foragers were given the opportunity to feed (BioGluc® solution) for 60 seconds every 40 minutes. After three hours on the flight mill the foragers were removed, placed in a 2ml Eppendorf tube, and sacrificed in a -20°C freezer. The bumblebees were then thawed for 1 hour the following day and thorax width measurements were taken 3 times using digital callipers (Mitutoyo Digimatic RS 600-880) and the mean value recorded, which is a standard measurement of bumblebee size (183).

### 2.2.5. Foraging arena insecticide exposure

Four colonies of *Bombus terrestris* (*Bombus terrestris audax* research hives containing 40-50 workers) were purchased from Biobest, Belgium and transferred into wooden nest boxes as already described. The four colonies were randomly allocated to four treatment groups (control, sulfoxaflor, flupyradifurone, or sulfoxaflor + flupyradifurone). A 1000ppm stock solution of flupyradifurone (PESTANAL<sup>®</sup>, analytical standard, Sigma Aldrich) was made up from 100mg of flupyradifurone in 100ml of acetone. 4ml was removed from 1L of BioGluc<sup>®</sup> and 4ml of the 1000ppm flupyradifurone stock solution was added, forming a BioGluc<sup>®</sup> solution of 4ppm flupyradifurone.

The control and the sulfoxaflor colonies were treated as described in section 2.2.3. The flupyradifurone colony was fed a BioGluc<sup>®</sup> solution with a flupyradifurone solution of 4ppm. The sulfoxaflor + flupyradifurone colony was fed a BioGluc<sup>®</sup> solution containing 4ppm of flupyradifurone and 5ppb of sulfoxaflor. All exposure periods lasted 14 days.

### 2.2.6. Foraging arena

Each colony was placed in an indoor flight arena (4.0m x 3.0m x 2.3m) maintained at 20°C and 50-60% relative humidity, with a day/night cycle of 12 hours, for 8 days. The nest box was kept dark and the entrance was opened by removing the metal divider on the first day, allowing the bees to fly freely within the flight arena. After an hour, a gravity feeder containing BioGluc<sup>®</sup> was placed 10cm from the colony. Every hour for the next 5 hours, the feeder was moved an additional metre away from the nest entrance, until the feeder was 5m from the nest. After every hour the feeder was observed for 5 minutes to ensure bumblebees were flying to and from the feeder. No observations were made during the first 24 hours to allow the foragers to acclimatise to the flight arena and learn their foraging route.

The following day, a video camera (Andoer 48.0 megapixel 4K) recorded and a visual observer counted the number of bumblebees leaving and returning to the nest. Observations took place for 1 hour between the hours of 9am and 11am and 1 hour between 1pm and 3pm. This was conducted for seven consecutive days. The footage was reviewed and cross-referenced with the observer's data.



This protocol was repeated for each colony. At the end of the experimental period, each bumblebee colony was sacrificed in a -20°C freezer.

#### 2.2.7. Data analysis

I performed exploratory data analysis to assess normality, homoscedasticity, and independence of data prior to statistical testing (184). All statistical analyses were performed using 'R' programming software version 4.1.1 (185). All figures were made using the ggplot2 package (186).

#### 2.2.8. Flight mill analysis

I produced a number of histograms to visualise the distribution of flight distance, flight duration, average speed, maximum speed and pause duration. The data was found to not adhere to a normal distribution. The histograms were plotted with log-transformed data against counts and against log-transformed counts. The residuals of each dependent variable were plotted and deemed to adhere to homoscedasticity.

The data was log-transformed to adhere to a normal distribution. To determine if there was a difference in flight parameters between the control and sulfoxaflor groups I conducted a MANCOVA with flight distance, flight duration, average speed, maximum speed and pause duration as continuous response variables and mean thorax width and colony as continuous and categorical covariates, respectively.

#### 2.2.9. Foraging arena analysis

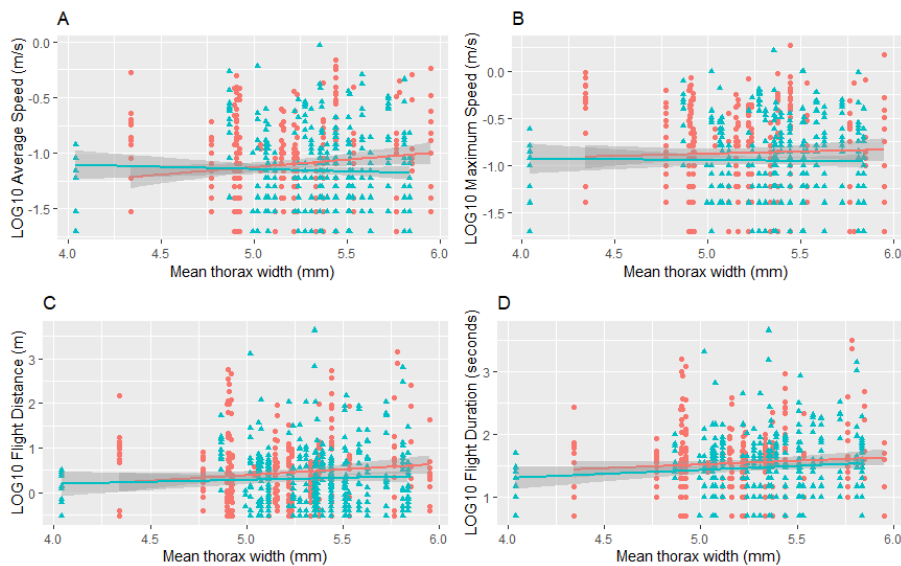
Data was log-transformed to adhere to a normal distribution. To determine if I could use the number of bees leaving the nest as a covariate to adjust for the difference in the number of bees returning to the nest I conducted an ANCOVA. The assumption being that the number of bumblebees returning to the nest is dependent on the number of bumblebees leaving the nest. I used bees leaving the nest as the response variable, the number of days post exposure as a blocking factor and presence or absences of pesticide and time of day (AM or PM) as treatment factors. The results showed a clear effect of day on number of bees leaving the nest, but no effects of any of the treatments, confirming that I could use the number of bees leaving the nest as a covariate.

I then conducted an ANCOVA with the number of bees returning to the nest as the response variable, the number of days post exposure as a blocking factor, number of bees leaving the nest as a covariate and time of day and presence or absence of sulfoxaflor and flupyradifurone as treatment factors. I also included two-way interactions between days and the presence or absence of sulfoxaflor and flupyradifurone and day and AM or PM.

## 2.3 Results

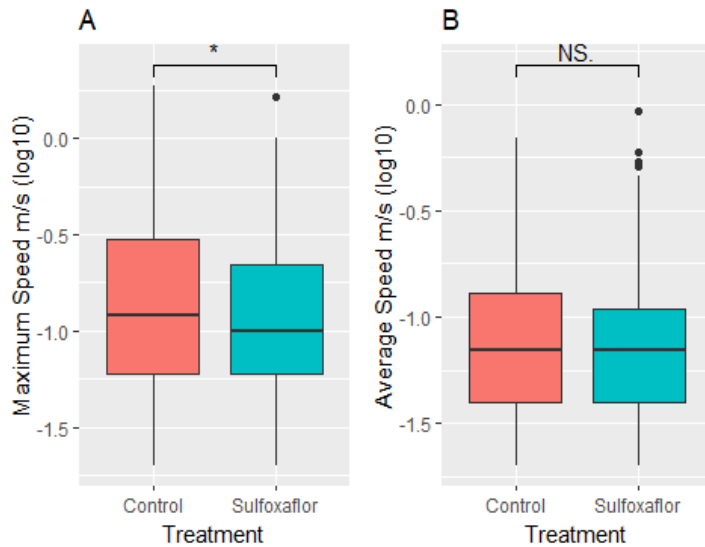
### 2.3.1. Flight mill

I found a significant difference in the flight distance travelled ( $F_{1, 712}=4.747$ ,  $p<0.05$ ) between the control and sulfoxaflor treated colonies, with controls travelling further. The flight distance was affected by bumblebee size, with larger bumblebees flying further, ( $F_{1, 712}=4.765$ ,  $p<0.05$ ) and colony ( $F_{8, 712}=6.497$ ,  $p<0.001$ ) (Fig. 2.3.1). The flight duration of bumblebees was also significantly affected by which colony they came from ( $F_{8, 712}=6.287$ ,  $p<0.001$ ) and body size, with larger bumblebees flying for longer, ( $F_{1, 712}=5.946$ ,  $p<0.05$ ) but not due to treatment ( $F_{1, 712}=3.282$ ,  $p=0.070$ ). The average speed of bumblebees was significantly



**Figure 2.3.1:** Scatterplot showing key flight characteristics (average (A) and maximum speed (B) in metres per second, distance flown in metres (C), and flight duration in seconds (D) against mean worker thorax width (mm) for both the control (red dots) and sulfoxaflor treated bumblebees (blue triangles).

affected by sulfoxaflor exposure ( $F_{1, 712}=4.267$ ,  $p<0.05$ ) and colony ( $F_{8, 712}=5.440$ ,  $p<0.001$ ), with the control group flying an average of  $0.112\text{ms}^{-1}$  compared with  $0.0947\text{ms}^{-1}$  for the sulfoxaflor group. Average speed was not affected by body size ( $F_{1, 712}=1.381$ ,  $p=0.240$ ). The



**Figure 2.3.2:** Boxplots of (A) maximum speed and (B) average speed versus treatment group. The box edges represent the inter-quartile range (IQR), the horizontal line represents the median and the error bars represent 95-percentile range. Outliers are plotted as filled black dots.

maximum speed was also significantly different between the sulfoxaflor and control groups ( $F_{1, 712}=6.665$ ,  $p<0.05$ ) with control and sulfoxaflor bumblebees having a mean maximum speed of  $0.221\text{ms}^{-1}$  and  $0.169\text{ms}^{-1}$ , respectively. There was a significant effect of colony on maximum speed ( $F_{8, 712}=5.627$ ,  $p<0.01$ ) but body size did not influence maximum speed ( $F_{1, 712}=0.083$ ,  $p=0.773$ ) (Fig. 2.3.2). Sulfoxaflor, thorax width nor colony affected the pause duration between successive flights.

### 2.3.2. Flight arena

I tested if I could use the number of bees leaving the nest as a covariate using an ANCOVA. I found a significant effect of day on the number of bees leaving the nest ( $F_{6, 42}=2.97$ ,  $p<0.05$ ), but no effect of any other treatment (Table 2.3.1).

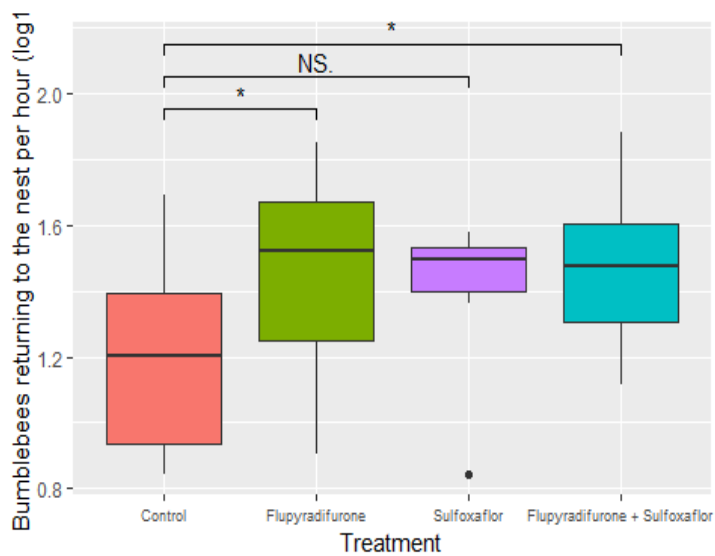
**Table 2.3.1:** Two-way analysis of covariance of bumblebee foragers leaving the nest.

Factor	Df	Sum of Squares	Mean of Squares	F value	P value
Flupyradifurone	1	0.262	0.262	2.220	0.144
Sulfoxaflor	1	0.077	0.077	0.653	0.424
AM.PM	1	0.047	0.047	0.395	0.533
Flupyradifurone:Sulfoxaflor	1	0.249	0.249	2.110	0.154
Flupyradifurone:AM.PM	1	0.000	0.000	0.000	0.987
Sulfoxaflor:AM.PM	1	0.020	0.020	0.173	0.680
Flupyradifurone:Sulfoxaflor:AM.PM	1	0.021	0.021	0.174	0.679
Day	6	2.100	0.351	2.970	0.017 *
Residuals	42	4.960	0.118		

I found a very highly significant effect of day ( $F_{6, 27}=21.8$ ,  $p<0.001$ ) and the number of bees leaving the nest ( $F_{1, 27}=348$ ,  $p<0.001$ ) on the number of bees returning to the nest (Table 2.3.2). I found a highly significant effect of time of day ( $F_{1, 27}=9.58$ ,  $p<0.01$ ). I also found a significant effect of flupyradifurone ( $F_{1, 27}=6.59$ ,  $p<0.05$ ) and sulfoxaflor ( $F_{1, 27}=5.24$ ,  $p<0.05$ ) on the number of bees returning to the nest (Fig. 2.3.3). There was no significant interacting effect between day and either pesticide nor between day and time of day.

**Table 2.3.2:** Two-way analysis of covariance of bumblebee foragers returning to the nest

Factor	Df	Sum of Squares	Mean of Squares	F value	P value
Bees leaving the nest $\log_{10}$	1	2.65	2.65	348	5.94e-17 ***
Day	6	0.997	0.166	21.8	3.57e-9 ***
Flupyradifurone	1	0.050	0.050	6.590	0.016 *
Sulfoxaflor	1	0.040	0.040	5.240	0.030 *
AM.PM	1	0.073	0.073	9.580	0.005 **
Flupyradifurone:Sulfoxaflor	1	0.0003	0.0003	0.029	0.867
Day:Sulfoxaflor	6	0.105	0.018	2.290	0.065 .
Day:Flupyradifurone	6	0.087	0.014	1.890	0.119
Day:AM.PM	6	0.033	0.006	0.729	0.630
Residuals	27	0.206	0.008		



**Figure 2.3.3:** The number of foragers returning to the nest per hour against treatment group. The box edges represent the inter-quartile range (IQR), the horizontal line represents the median and the error bars represent 95-percentile range. Outliers are plotted as filled black dots.

## 2.4 Discussion

I demonstrate that chronic exposure to conservative, field-realistic levels (5ppb) of the sulfoximine-based pesticide, sulfoxaflor, caused a reduction in the average and maximum flight speed of *Bombus terrestris* audax workers and an increase in distance flown per flight as assessed by flight mills. However, at the applied dosages, sulfoxaflor did not affect flight duration or the duration of pauses between successive flights.

### 2.4.1. Flight mill

Bumblebees from colonies chronically exposed to sulfoxaflor for 2 weeks had a reduced average and maximum flight speed (Fig. 2.3.2), both important factors for foraging success. A

reduction in flight speed carries high energetic costs for flying insects (187). Energy consumption is at the heart of all bumblebee foraging decisions (188), thus flight speed is an ecologically relevant feature of flight performance in natural environments. My findings contrast with those of a similar study on flight mills which demonstrated acute neonicotinoid exposure resulted in bumblebees flying faster than controls (165). In their paper, Kenna *et al* found that while exposed bumblebees flew faster than controls, flight stamina was reduced and exposed bumblebees flew for a shorter duration than controls. They suggest that acute exposure may not affect immediate motor function but rather flight stamina. In a more recent study, Kenna *et al.* reported that chronic exposure to sulfoxaflor (10ppb) over a period of 6 days did not result in a significant difference in average or maximum flight speed of tethered bumblebees (189). Previous studies on the neonicotinoid imidacloprid have shown that it causes rapid mitochondrial depolarization in the neurons of honeybees and bumblebees, resulting in a reduction in mitochondrial activity and impairment of respiratory processes (190, 191). This may explain the observed reduction in speed in acutely treated bumblebees, as neonicotinoids and sulfoxaflor have a similar mechanism of action and target the same nicotinic acetylcholine receptors (166, 168). This could also be causing a reduction in flight speeds due to the impact sulfoxaflor has on development. However, in the present study, the age of workers was not controlled or known when attached to the flight mill. Previous research has shown that a 2-week chronic exposure of sulfoxaflor at 5ppb results in a 54% reduction in reproductive output and can impair larval growth (176, 177). Whether due to physiological or developmental factors, a reduction in flight speed and hence an increase in energy consumption would have negative impacts on individual and colony-level fitness.

The existing literature suggests that sulfoxaflor does not impair bee behaviour, although the available data is limited (176, 192). The results from my flight mill experiment show that chronic sulfoxaflor exposure does not affect the flight duration or time between successive flights of bumblebees, but it does reduce flight speed while increasing the distance travelled per flight. My findings do not contradict or support previous studies showing no long-term impact of chronic sulfoxaflor exposure on bumblebee foraging success (176). Notably, similar findings were observed in experiments on locusts (*Locusta migratoria*), which found no effect of acute sulfoxaflor exposure on behaviour, suggesting that this finding may be common across insect taxa (193). But my findings contrast with a recent study that showed chronic

exposure at double the dose (10ppb) I used did not significantly affect the flight speed of tethered bumblebees (189).

However, the flight mill data in this chapter is limited by a small sample size ( $n=50$ ) and therefore has a high likelihood of type II error. The sample size per colony in this study ( $n=5$ ) is not representative of an average bumblebee colony, which can exceed two-hundred and fifty individuals (194). Therefore, in future, the impact of sulfoxaflor on bumblebee flight characteristics should be re-assessed using a larger, more representative sample size.

#### 2.4.2. Foraging arena

I present, for the first time, the findings of a study on the combined impacts of sulfoxaflor and flupyradifurone on bumblebee foraging activity. My data show bumblebee colonies treated with flupyradifurone and sulfoxaflor, alone and together, had a higher number of foragers returning to the nest (Fig. 2.3.3). In this experiment, the number of foragers leaving and returning to the nest are a proxy for foraging activity. I used the number of foragers leaving the nest as a covariate in the analysis because it stands to reason that the number of bees returning to the nest is reliant on the number of bees leaving the nest (see methods). An increase in foraging activity is also seen in colonies exposed to neonicotinoid pesticides (104). This similar behaviour may be explained by the shared mode of action between sulfoxaflor, flupyradifurone, and neonicotinoids. An effect of these pesticides being agonists of insect acetylcholine receptors is acutely increased neuronal activity (195, 196). The hyperactivity effect of neonicotinoids may be shared by sulfoxaflor and/or flupyradifurone and explain the behaviour observed here. This effect has been previously suggested as an explanation for neonicotinoid impacts on honeybee flight and locomotor activity (197, 198). Future work should investigate the impact of these two pesticides on flight and locomotor activity in bumblebees to determine similar effects on flight speed and distance travelled per flight are observed, as seen in honeybees exposed to neonicotinoids.

To my knowledge, three papers have assessed the impact of flupyradifurone on bumblebees and only one focuses on behaviour (199-201). Siviter and Muth (2022) assessed the sub-lethal impact of flupyradifurone on bumblebee behaviour. The authors found acute, field realistic

exposure (4ppm) impaired olfactory learning, colour learning and most interestingly, reduced individual's motivation to feed when presented with sucrose water (201). A previous study has shown bumblebees treated with sulfoxaflor had a lower frequency of flower visitations but had no effect on the daily number of foraging flights per colony (202). What is clear from the existing literature is that these two agrochemicals impact foraging behaviour in some way, but, the details of how and to what extent, are not yet clear. From my findings, I can say that sub-lethal chronic exposure of flupyradifurone, and sulfoxaflor, may affect the foraging frequency of bumblebees.

There is potential conflation of my result of a difference of foraging activity due to treatment and due to colony. Each treatment group contained a single colony and, thus, the difference in foraging activity I observe could be explained by a difference in activity between colonies, of which there is considerable existing evidence to support (203-206). Additionally, my finding of significantly different numbers of bumblebees returning to the nest between the morning and afternoon may be explained by natural variation in foraging activity during the day. *B. terrestris* and *Bombus pascuorum* both share a pattern of daily foraging activity, with a peak of foragers in the early morning (5am-9am) and early evening (4pm-6pm) (206). This difference in natural foraging activity between the morning and afternoon could be the explanation for the observed difference in activity during the day especially as there is no significant interaction between treatment group and time of day (Table 2.3.1).

## 2.5 Conclusion

As bumblebees are central place foragers, they must travel to key resources and then travel back to the nest. A change in the frequency of these trips or a reduction in speed during these trips could have detrimental energy costs associated with them resulting in an energetic deficit for individuals and the colony as a whole. My study suggests the butenolide pesticide, flupyradifurone, and the sulfoximine-based pesticide, sulfoxaflor, may be influencing these key characteristics of *B. terrestris*. However, caution should be exercised due to the conflation of treatment and colony in this study. Future work should aim to unravel these two components with a higher number for colonies per group and continue to investigate the



potential interactions these chemicals have with one another on normal bumblebee behaviour.

## **Chapter 3**

The impact of sulfoxaflor and  
flupyradifurone on *Bombus terrestris*

# flight characteristics assessed by flight mill

## 3 Chapter 3: The impact of sulfoxaflor and flupyradifurone on *Bombus terrestris* flight characteristics assessed by flight mill

### 3.1 Introduction

Bee population declines are driven by numerous anthropogenic stressors, including habitat loss, intensive agriculture, parasitic infection, and climate change, as well as the interaction between stressors (33, 207-209). One key driver of declines is exposure to agrochemicals (e.g. insecticides, herbicides, or fungicides) such as neonicotinoid pesticides (32, 210, 211). Neonicotinoids are the most commonly used insecticide globally (212). These chemicals are designed to control insect numbers by acting as agonists of nicotinic acetylcholine receptors (nAChRs), and they are applied most frequently as either a seed treatment or as a foliar spray, allowing for effective use across a broad range of crops (173). As systemic pesticides, they are expressed in all tissues of treated plants, including the pollen and nectar of flowering species (32, 173, 213) where foraging bees may be exposed. Field-realistic concentrations of neonicotinoids have been proven to significantly negatively impact bees and other pollinators (32, 214-216). This evidence led to restrictions on the outdoor agricultural use of three neonicotinoid pesticides (clothianidin, imidacloprid, and thiamethoxam) in the European

Union (EU) (217). Outside of the EU, there has been an increase in neonicotinoid resistance and, with it, a demand for novel insecticides to control pest-species resistance (213, 218).

Two neonicotinoid alternatives that have been registered globally for agricultural use are flupyradifurone and sulfoxaflor. These alternatives are commonly applied either as a spray or as a seed treatment, and they share the same mode of action as neonicotinoids (166, 167). However, these novel insecticides differ in their chemical structure to neonicotinoids, specifically in their structural activity relations (166-170, 219). Although flupyradifurone, sulfoxaflor, and neonicotinoids all share a similar mode of action, they are classified into different groups by the Insecticide Resistance Action Committee. Specifically, neonicotinoids are in group 4A, sulfoxaflor is in group 4C, and flupyradifurone is in group 4D (166, 167)) and as such do not fall under the same EU restrictions as neonicotinoids. Flupyradifurone and sulfoxaflor have been shown to effectively control pest species that have developed resistance to neonicotinoids (167, 168, 220), making them promising candidates for replacing neonicotinoids in regions where pest resistance is prevalent (221).

Environmental Risk Assessment (ERA) schemes are based on the premise of a single stressor (agrochemicals in this case) on a single crop paradigm. However, ERA schemes largely overlook how interactions between stressors affect bee health at the landscape scale (222, 223). Indeed, a recent meta-analysis suggests that ERA schemes underestimate the interactive effect anthropogenic stressors have on bee mortality, especially agrochemicals (33). Toxicity to bumblebees is not a requirement in current pesticide regulatory testing despite the fact that some wild bee species are >2,500 times more sensitive to agrochemicals than other managed bee pollinators (144). This raises the concern that, like neonicotinoid pesticides, both novel pesticides may have effects on non-target species, particularly wild bees.

The present study assesses the impact of chronic field-realistic exposure to two novel insecticides, alone and in combination, on a key pollinator species' (*Bombus terrestris*) flight characteristics and propensity to fly. I hypothesized exposure to the insecticides sulfoxaflor and flupyradifurone may negatively affect bumblebee flight performance based on the limited literature on the impact of these pesticides (199, 224).

## 3.2 Methods

### 3.2.1. Bumblebee colonies

Eight *B. terrestris* colonies (*Bombus terrestris audax* research hives containing 40-50 workers) were purchased from Biobest, Belgium and placed in the lab at the Rothamsted Research Experimental Farm on 26<sup>th</sup> April 2021. On arrival, colonies were transferred into wooden nest boxes (210mm x 294 mm x 181mm) with a Perspex lid and tunnel attached to allow easy access for feeding and extracting individual bumblebees. The colonies were transported into the flight mill laboratory maintained at 22°C with 50-60% relative humidity and a 16-8 hour light-dark cycle. Colonies were fed honeybee collected pollen (Agralan Growers) *ad libitum*.

### 3.2.2. Insecticide exposure

Two colonies were randomly allocated to each of the four treatment groups: control, sulfoxaflor, flupyradifurone or sulfoxaflor + flupyradifurone. Sulfoxaflor and flupyradifurone solutions were prepared as described in section 2.2.3 and 2.2.5, respectively. All colonies in this experiment were exposed in the same way as described in section 2.2.3 and 2.2.5.

Foraging bumblebees fly to an average distance of 2.2km from the nest (160-162) meaning that foraging bees visit an area of approximately 15.2km<sup>2</sup> around the colony. The average size of a farm in Europe is 0.17 km<sup>2</sup> (225), thus a foraging distance of 15.2km<sup>2</sup> is easily covered by a variety of crops, exposing a colony to multiple pesticides used for different crops in a rural environment. Additionally, multitudes of pesticides are available for agricultural use, for example, European farmers have access to more than 450 different active ingredients (226), including sulfoxaflor and flupyradifurone. Hence, there is a possibility that bumblebees could be exposed to both of these agrochemicals.

### 3.2.3. Flight mill

Fifty worker bumblebees were haphazardly selected from each colony to be attached to the flight mills (total n=400). Each colony was allocated a number that corresponded to the side of an eight-sided die. The die was rolled and a bumblebee was selected from the colony that corresponded to the number displayed on the die. If the die showed the number of a colony that had already reached the 50 bumblebee quota, the die was rerolled. Selected bumblebees were removed from the Perspex tunnel with forceps and a metal loop, shaped from

0.25mmx0.5mm steel wire, was attached using an adhesive (Gorilla Super Glue Gel) on the dorsal side of the thorax. Individuals were held in place for ten minutes to allow the adhesive to bond before being placed in a wooden nest box with a Perspex lid for an additional ten minutes to acclimatise to the loop attachment. From the experiment in Chapter 2, I found that bumblebees immediately attached to the flight mill apparatus after loop attachment were less likely to fly within the first ten minutes. To reduce the likelihood of bumblebees not flying because of the stress of loop attachment I added the acclimatisation period to my protocol. Bumblebees were attached to the flight mill for 1 hour. From the experimental work in Chapter 2, I found that after an hour of being on the flight mill bumblebees would slow and fly less frequently. Additionally, 1 hour more closely matches the average flight time for free flying bumblebees than 3 hours (227).

Seven flight mills were used simultaneously. After an hour on the flight mills individuals were removed and placed in labelled Eppendorf tubes and sacrificed at  $-20^{\circ}\text{C}$  in a freezer. Bumblebees were thawed the following day and thorax width measurements were taken using digital callipers (Mitutoyo Digimatic RS 600-880) and the mean value calculated.

#### 3.2.4. Data analysis

The Akaike information criterion and maximum likelihood estimation analyses were performed using bespoke Fortran 77 code. All other statistical analyses were performed using 'R' programming software version 4.1.1 (185) and the 'lme4' (228) package. All figures were made using the ggplot2 package (186).

##### 3.2.4.1. Flight mill

Several histograms were produced to visualise the distribution of the data and found that it did not adhere to a normal distribution. The data was subsequently log transformed.

A linear model was used to compare variation in mean thorax width (body size) between treatments, with treatment as the only fixed effect. I conducted a TukeyHSD pairwise comparison test to identify if any observed differences in flight characteristics between the treatment groups was statistically significant. A *post hoc* Tukey-HSD pairwise comparison was conducted for each response variable: flight distance, flight duration, maximum speed, average speed, and pause duration with treatment as the explanatory variable and colony as

a covariate. This type of statistical test was designed, and its significance levels established, without regard to the overall F value (229). As such, this test can be conducted without a prior significance group effect from a global test (e.g ANOVA). The name of these kinds of pairwise comparisons, *a posteriori*, is interpreted as ‘after the F-test’ but it should be interpreted as ‘without any *a priori* hypotheses’.

#### 3.2.4.2. Detection of power-law distributions

I tested for evidence of a pervasive ‘idling activity’ template in the flight patterns of bumblebees tethered to the rotational flight mill, following the approach of Reynolds *et al.* (2007) (230) using the raw, non-transformed data. This ‘idling activity’ has previously been observed in the European honeybee (*A. mellifera*) and multiple species of noctuid moth tethered to flight mills exposed to minimal visual cues, and in individual fruit flies (*Drosophila melanogaster*) moving within featureless unchanging arenas (231-233). In the absence of a stressor, the time an animal spends moving and remaining stationary are typically power-law distributed over a range of scales of magnitude and characterised by exponents close to 3/2 (234-237). These characteristics of movement and pause duration are not expected to be present when individuals are stressed. I wanted to know if bumblebees exposed to sulfoxaflor and/or flupyradifurone would behave as if stressed in relation to their flight and pause durations. Following a well-established protocol (238), flight-length and pause duration distributions were fitted to power-law distributions (indicative of Lévy flights) and to exponential distributions (a null distribution) using maximum likelihood methods (239).

These model distributions are prescribed by:

$$p_1(l) = N_1 l^{-\mu} \quad (1a)$$

$$p_2(l) = N_2 \exp(-\lambda l) \quad (1b)$$

Where the constants  $N_1$  and  $N_2$  ensure that the frequency distributions sum correctly to unity when integrated over all flight or pause lengths between the lower and upper cut-offs,  $\mu$  is the power-law exponent and  $\lambda$  is the exponential decay rate. The lower cut-off was taken to be 10 seconds and the upper cut-off was taken to be the duration of the longest flight in seconds. The best-fit distribution was identified using the Akaike information criterion, the

outcomes of which, the Akaike weights, range from 0 (no support for the model) to 1 (full support for the model).

#### 3.2.4.3. Propensity to fly

I conducted a logistical regression in the form of a generalized linear mixed model (GLMM) under a binomial family distribution with the propensity to fly ( $y/n$ ) as the response variable, treatment as the explanatory variable, and thorax width and colony as random effects. The usefulness of the model was deemed satisfactory by calculating the chi squared statistic and associated p value ( $<0.05$ ) from the null and residual deviance of the model.

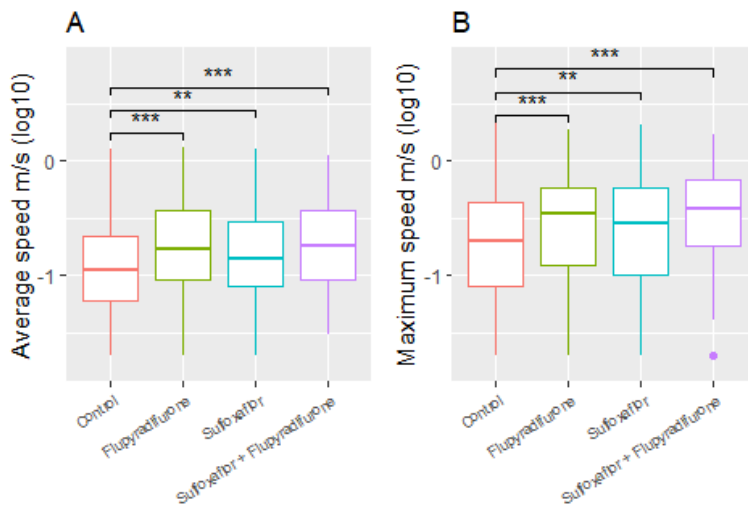
The variance in the data explained by the random effect of colony was not significant, so it was eliminated to simplify the model, returning it to a generalized linear model (GLM).

### 3.3 Results

#### 3.3.1. Flight mill

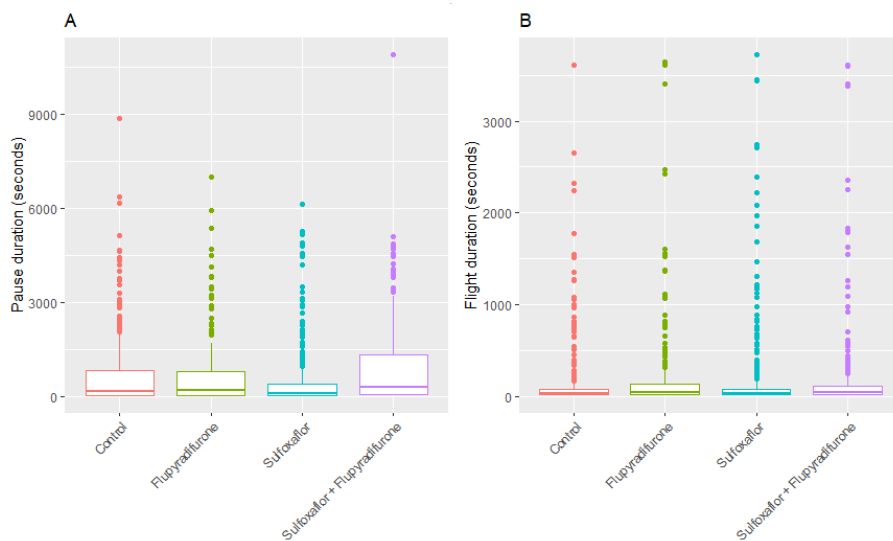
The results from the *post hoc* TukeyHSD pairwise comparison showed the average speed of bumblebees in all three treatment groups was higher than control groups: flupyradifurone ( $M=0.263$  m/s,  $SE=0.254$ ); sulfoxaflor ( $M=0.227$  m/s,  $SE=0.229$ ); flupyradifurone and sulfoxaflor ( $M=0.261$  m/s,  $SE=0.223$ ); and controls ( $M=0.191$  m/s,  $SE=0.224$ ) (Fig. 3.3.1). The maximum speed of bumblebees was also higher in all three treatment groups [flupyradifurone ( $M=0.432$  m/s,  $SE=0.386$ ), sulfoxaflor ( $M=0.408$  m/s,  $SE=0.394$ ), flupyradifurone and sulfoxaflor ( $M=0.471$ m/s,  $SE=0.371$ )] compared with the control group ( $M=0.352$  m/s,  $SE=0.398$ ) (Fig. 3.3.1). Bumblebees treated with flupyradifurone alone ( $M=127$  m,  $SE=389$ ) and in combination with sulfoxaflor ( $M=118$  m,  $SE=392$ ) flew significantly further compared with control colonies ( $M=70.6$  m,  $SE=276$ ). The pause duration between successive flights was significantly longer in bumblebees treated with both flupyradifurone and sulfoxaflor ( $M=937$  sec,  $SE=1380$ ) compared with bumblebees from the control group ( $M=$

716 sec, SE=1240). Flight duration was not significantly different between treatment and control groups (Fig. 3.3.2).



**Figure 3.3.1:** Boxplot of  $\log_{10}$  average speed (A) and maximum speed (B) versus treatment. Data points represent individual flight bouts. The box edges represent the inter-quartile range (IQR), the horizontal line represents the median and the error bars represent 95-percentile range. Outliers are plotted as filled coloured dots.





**Figure 3.3.2:** Boxplot showing the effect of treatment on flight (A) and pause duration (B) between successive flights. The box edges represent the inter-quartile range (IQR), the horizontal line represents the median and the error bars represent 95-percentile range. Outliers are plotted as filled dots. Data points represent individual flight bouts.

### 3.3.2. Power-law distributions

I demonstrate that there is very clear evidence of a power law in the flight duration and pause duration data. The Akaike weight (weight of evidence of the power law being the best model) is 1.00, indicating complete support for the power law. The maximum likelihood for the power law exponent (the power) is 1.53. The control group is characterized by a power law with exponent 1.62 for flight duration, which is not significantly different from the treatment groups (flupyradifurone 1.51, sulfoxaflor 1.55, flupyradifurone and sulfoxaflor 1.62). The power law exponent of the control group for pause duration is 1.37, which is also not significantly different from the treatment groups (flupyradifurone 1.36, sulfoxaflor 1.32, flupyradifurone and sulfoxaflor 1.37). Although not significant, the largest departure from the expected  $3/2$  value for flight durations occur for flupyradifurone and sulfoxaflor. Bumblebees treated with sulfoxaflor, flupyradifurone and both in combination, all behave normally in terms of flight and pause duration (Fig. 3.3.2).

### 3.3.3. Propensity to fly

Flight was initiated by 317 workers, comprising 68% of control (n=68 of 100); 73% of flupyradifurone workers (n=73 of 100); 91% of sulfoxaflor workers (n=91 of 100); and 85% of

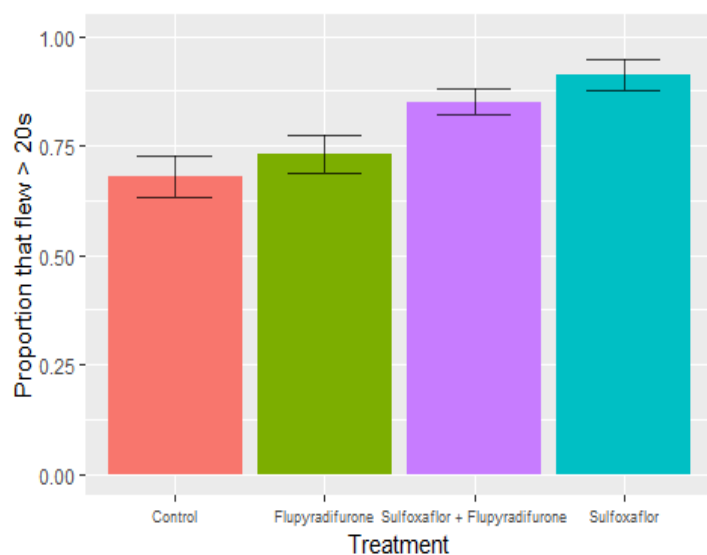
flupyradifurone and sulfoxaflor combined workers (n=85 of 100) (Fig. 3.3.3). Workers in the sulfoxaflor group (sulfoxaflor:  $Z=3.544$ ,  $p<0.001$ ) and the flupyradifurone and sulfoxaflor combined groups ( $Z=2.847$ ,  $p<0.01$ ) had a significantly higher likelihood of flying (Table 3.3.1). The linear model found bumblebees had a significantly larger body size in the sulfoxaflor and flupyradifurone combination treatment ( $t=-2.438$ ,  $p=0.015$ ) compared with other treatment groups (Table 3.3.2). I found thorax width was not a significant predictor of propensity to fly ( $z=1.193$ ,  $p=0.233$ ; Table 3.3.2).

**Table 3.3.1:** Generalised linear model summary output for the effect of pesticide (flupyradifurone, sulfoxaflor and sulfoxaflor + flupyradifurone) exposure and worker body size (thorax width) on the propensity of individuals to initiate flight

		Estimate	Std.Error	z value	P value
<b>Propensity to fly (n=400)</b>	Intercept	-1.572	1.979	-0.794	0.427
	Treatment - Flupyradifurone	0.240	0.312	0.770	0.441
	Treatment – Sulfoxaflor	1.451	0.410	3.544	<b>&lt;0.001</b>
	Treatment –Sulfoxaflor+ Flupyradifurone	1.008	0.354	2.847	<b>0.004</b>
	Thorax width	0.451	0.378	1.193	0.233

**Table 3.3.2:** Linear model summary output for the relationship of pesticide group (flupyradifurone, sulfoxaflor and sulfoxaflor + flupyradifurone) and worker body size (thorax width)

		Estimate	Std.Error	t value	Pr(> t )
<b>Mean thorax width (n=400)</b>	Intercept	5.2224762	0.0322523	161.926	<b>&lt;0.001</b>
	Treatment - Flupyradifurone	0.0001293	0.0464190	-0.003	0.9978
	Treatment – Sulfoxaflor + Flupyradifurone	0.1125762	0.0461783	-2.438	<b>0.0152</b>
	Treatment –Sulfoxaflor	0.0167174	0.0470600	0.355	0.7226



**Figure 3.3.3:** Barplot of the effect of treatment on the proportion of bumblebees that initiated flight ( $n=100$  per group). Error bars represent  $\pm$  SEM.

### 3.4 Discussion

The present study is the first to assess the combined impacts of sulfoxaflor and flupyradifurone on bumblebee flight properties. The findings demonstrate that chronic, field-realistic exposure to flupyradifurone, sulfoxaflor, and both in combination, increase average and maximum flight speed. It also demonstrates that flupyradifurone alone and in combination with sulfoxaflor increases the distance flown by bumblebees when compared with controls. From the power-law calculations, I found that bumblebees from all treatment groups behaved the same as control bumblebees in terms of their flight and pause durations. However, the pause duration between successive flights was significantly longer for bumblebees exposed to a combination of flupyradifurone and sulfoxaflor, when determined by TukeyHSD *post hoc* test. Bumblebees exposed to sulfoxaflor alone and in combination with flupyradifurone had an increased propensity to fly but flupyradifurone alone had no measurable effect.

### 3.4.1. Flight mill

Bumblebees chronically exposed for 2-weeks to sulfoxaflor (5ppb) and flupyradifurone (4ppm), separately and together, have an increased average and maximum flight speed (Fig. 3.3.1). An explanation for the observed increase in the average and maximum flight speed is that certain flight characteristics are affected by sulfoxaflor and flupyradifurone exposure. However, to date no studies have tested this. There are a small number of studies on the impact of neonicotinoids on bee flight characteristics. Acute exposure to thiamethoxam in honeybees increases mean flight velocity (240) but chronic exposure (1-2 days) reduces the mean velocity by 7% (197). In bumblebees, acute exposure to imidacloprid causes a significant increase to average velocity (165).

As previously mentioned sulfoxaflor and flupyradifurone share a similar mode of action with neonicotinoid pesticides (166, 167). Neonicotinoids are agonists of acetylcholine receptors (241) and can acutely increase neuronal activity at field relevant levels (195, 196). A consequence of this may be hyperactivity, which would explain the observations of higher velocities in workers treated with sulfoxaflor and/or flupyradifurone. This phenomenon has previously been suggested as an explanation for neonicotinoid effects on honeybee flight locomotor activity (197, 198, 242, 243). I found bumblebees exposed to flupyradifurone alone and in combination with sulfoxaflor have a higher mean and maximum speed compared with sulfoxaflor alone, suggesting that flupyradifurone may be facilitating higher stimulation and hence hyperactivity compared to sulfoxaflor alone. A potential cost to this observed hyperactivity could be reduced flight distance and duration due to energy depletion and faster muscle fatigue (165), but further testing is needed to fully understand this.

In contrast, bumblebees exposed to flupyradifurone alone and together with sulfoxaflor flew significantly further than controls. Suggesting energy depletion and muscle fatigue, from flying faster, is not common to these pesticides as it is with the neonicotinoid imidacloprid (165). Flying further and faster is likely detrimental. Neonicotinoids have been proven to cause flight disorientation (163, 243, 244). Additionally, at the same dosage used in this study, flupyradifurone has been found to impair visual and olfactory learning and memory (201), which could hinder the bees' ability to navigate. Bees that fly further from their nest and more erratically have a lower likelihood of returning to the nest (245). A decline in the number of

neonicotinoid treated honeybees returning to their nest has been demonstrated at the colony level, in two separate studies (35, 246). Therefore, it is possible that bumblebees treated with sublethal doses of flupyradifurone and/or sulfoxaflor could experience reduced homing ability, which would have detrimental effects on the colony.

#### 3.4.2. Power-law distributions

Bumblebees exposed to flupyradifurone and sulfoxaflor, separately and together, behaved normally in respect to their pause duration between successive flights and flight durations. The power-law distributions of flight and pause durations of these three groups have an exponent close to  $3/2$ , which is indicative of a null flight pattern; seen in healthy invertebrates flying in the absence of external stimuli (231). This null pattern is not expected in stressed bumblebees because the power-laws are lost when animals are stressed (234-237). Many animal species take pauses during their movements, which are thought to provide various energetic benefits and improve endurance by aiding in recovery from fatigue (247, 248).

Here, I demonstrate for the first time that decision-making regarding pauses and flight durations in a key pollinator species are unaffected by chronic exposure to sulfoxaflor (5ppb) and flupyradifurone (4ppm), alone and in combination. This aligns with previous work on honeybees exposed to the gut parasite *Nosema ceranae* finding search patterns unaffected by infection (146). My findings support the theory that environmental stressors do not impact the fundamental characteristics of the neuronal and sensory components involved in bumblebee decision-making processes.

#### 3.4.3. Propensity to fly

Bumblebees exposed to sulfoxaflor alone and in combination with flupyradifurone had a higher propensity to fly than controls. A higher propensity to fly may be considered a positive trait if this behaviour translates into an increase in foraging activity. However, sulfoxaflor has been shown to reduce the number of flower visitations in bumblebees (202) and flupyradifurone impairs olfactory and visual learning and memory in bumblebees (201). Therefore, although bumblebees may be more likely to initiate flight it could result in reduced foraging and homing ability due to impairment of memory retention and subsequent disorientation. Combined with the findings in the present study of bumblebees flying faster

and further, an increased propensity to fly when exposed to these chemicals could result in a potential energy deficit for the colony, and loss of foragers that do not return to the nest, as seen in honeybees exposed to sulfoxaflor (249).

There is a high likelihood of pollinators being exposed to multiple pesticides in agricultural environments (155, 250, 251), with most agrochemicals interacting synergistically and increasing their combined impact on bee mortality (33). Given the growing global demand for neonicotinoid alternatives, and current lack of data surrounding the impact novel agrochemicals have on key pollinator species, I recommend ERA schemes consider the likely interacting effects of current and future agrochemicals on key pollinator species at lethal and sub-lethal dosages.

#### 3.4.4. Conclusion

Flying, and by extension, foraging, is an energetically costly behaviour that is essential for central place foraging species such as the bumblebee. Changes to flight characteristics, such as speed, distance and pause duration, will have appropriate energetic costs associated with them. In this chapter, I demonstrate that prolonged exposure (over 14 days) to sulfoxaflor and flupyradifurone, either individually or in combination, led to a significant impact of flight characteristics of bumblebees attached to flight mills. A change in flight performance would logically affect the energy costs of each foraging bout. Therefore, the forager would experience a different energetic cost than non-exposed bumblebees, affecting individual foraging rates and, ultimately, colony level resources and colony health.

In contrast with my results, a recent study found that chronic exposure to sulfoxaflor for six days at double the dose used here (10ppb) had no significant impact on the average or maximum flight speed in bumblebees (189). Given that this study employed a higher dosage compared to the present study, one would expect to observe either similar or more significant increases in the average and maximum flight speed of tethered bumblebees. However, since this is not the case, the only remaining possibility is the difference in exposure time used, which was six days compared to our fourteen days. Nevertheless, this disparity in results necessitates further empirical investigation.

This chapter provides additional evidence to the growing concern over sublethal affects of pesticides on key pollinator species as well as the under-studied effects of the novel neonicotinoid replacements, sulfoxaflor and flupyradifurone on non-target insects.

## **Chapter 4**

The impact of flupyradifurone and boscalid on *Bombus terrestris* flight characteristics assessed by flight mill



## 4 Chapter 4: The impact of flupyradifurone and boscalid on *Bombus terrestris* flight characteristics assessed by flight mill

### 4.1 Introduction

Agrochemicals are used in agricultural environments to enhance yield and control pests (252, 253). The three most commonly used agrochemicals are insecticides, herbicides, and fungicides and are often used in conjunction (254, 255). Global agrochemical use has doubled since 1990, with many chemicals having detrimental effects on non-target beneficial organisms such as bees (210, 255, 256). A stringent risk assessment process on these chemicals must be conducted before being authorised for commercial use. However, current risk assessment procedures only test single compounds and do not account for multiple pesticide scenarios which bees are exposed to in agricultural environments (155, 250). Exposure to multiple chemicals may lead to synergistic effects in bees (33). Additionally, multiple bee species have differing levels of sensitivity to single (63) and multiple pesticides (257, 258) highlighting the necessity for pesticide risk assessment schemes to include bee species other than *Apis mellifera* (222, 259).

Policy-makers have paid considerable attention to pesticides because of their harmful effects on bees, but the focus of this attention has primarily been on insecticides. A small number of papers have evaluated the impact of non-insecticide agrochemicals on bees. For example, herbicides have been shown to affect bee navigation, learning, and larval development (260-262) and fungicides have been shown to influence food consumption, metabolism, and larval development (263-265) [see (266) for a review on fungicides and bees]. The most commonly detected agrochemical in free-flying wild bumblebees in an agricultural landscape is the fungicide boscalid (66).

Boscalid and other fungicides are commonly used in a prophylactic manner and may be applied up to 10 times per season in some cases (267). In contrast with other agrochemicals, fungicides are generally considered not acutely toxic to bees (268), and are often sprayed during the flowering season to control for fungal diseases (269). At field-realistic doses,

boscalid reduces the wingbeat frequency of honeybee workers in flight (270) and, when in formulation with another fungicide, pyraclostrobin (Pristine®), induces earlier foraging and reduces worker honeybee longevity (271). Fungicides may also have synergistic effects when combined with other agrochemicals, thereby enhancing their toxicity to bees (33).

Flupyradifurone is a novel agrochemical that is licensed for agricultural use. Flupyradifurone at high dosages increases larval mortality in the Asian honeybee (*Apis cerana*) and impairs olfactory learning and memory at low and high doses in adult honeybee workers (272). Flupyradifurone has already been shown to be more toxic in the presence of other agrochemicals (224, 273), including in the presence of another fungicide, increasing honeybee mortality (274). As boscalid is the most commonly identified agrochemical in free-flying, wild bumblebees, there is a high likelihood that bumblebees will be exposed to both flupyradifurone and boscalid.

In this study, I assess, for the first time, the impact on *Bombus terrestris* flight characteristics and behaviour to chronic exposure of field-realistic levels of the butenolide-based pesticide, flupyradifurone and the carboximide class fungicide, boscalid, on their own and in combination. The majority of papers assessing the impact of fungicides on bees look at mortality and not sublethal effects (275). I address this knowledge gap by testing the following hypotheses: (i) the agrochemicals flupyradifurone and boscalid impact flight characteristics of *B. terrestris* workers; and (ii) decision making of *B. terrestris* is impacted by these agrochemicals.

## 4.2 Methods

### 4.2.1. Bumblebee colonies

Eight *B. terrestris audax* colonies (*Bombus terrestris audax* research hives containing 40-50 workers) were purchased from Biobest, Belgium and were transported to the Rothamsted Research Experimental Farm laboratory on the 28<sup>th</sup> July 2021. Colonies were transferred to wooden nest boxes and maintained as described in Chapter 2 section 2.2.1.

#### 4.2.1 Agrochemical exposure

Two colonies were randomly allocated to each of the four treatment groups: control, flupyradifurone, boscalid or flupyradifurone + boscalid. Colonies from the flupyradifurone and control groups were treated in the same way as described in Chapter 2 section 2.2.5. and 2.2.3, respectively.

Boscalid is a commonly used fungicide of the carboxamide family with a broad-spectrum activity, acting by inhibiting fungal respiration (276). It is applied to several crops including oilseed rape, grapevines, cereals, and in fruits and vegetables such as carrots cabbages, and beans, mainly during the plant growth and flowering period. Like many agrochemicals, boscalid has been detected in wild pollinators, being the most commonly detected in bumblebees in agricultural and urban landscapes (66). Nectar residues of boscalid have been described at 1.43 ppm (277), but concentrations of up to 440ppb in nectar and 60,500ppb in pollen have been recorded in blooming cherry trees (278). I chose 25ppb as the dosage for the experiment due to the residual levels of boscalid in nectar seven days post foliar spray (266), so the dosage here is conservative and field-realistic.

Boscalid has a solubility in water of 4mg/L and 180g/L in acetone. To enable full solution, boscalid (Sigma-Aldrich, batch number BCCF3773,  $\geq 98.0\%$  purity) was diluted in acetone. A stock solution was made from 100mg of boscalid dissolved in 100ml of acetone at a concentration of 1000ppm. The stock solution was diluted to 1000ml with acetone to create a solution with a concentration of 100ppm. 25 $\mu$ l was removed from 1L of BioGluc<sup>®</sup> and 25 $\mu$ l of the 100ppm boscalid solution was then added to 1L of BioGluc<sup>®</sup> to create a 25ppb boscalid solution.

The flupyradifurone + boscalid colonies were given a BioGluc<sup>®</sup> solution containing 25ppb boscalid and 4ppm flupyradifurone for 2 weeks.

#### 4.2.2 Flight mills

Bumblebees were prepared for attachment to the flight mills as described in section 3.2.3. Fifty workers were selected from each colony; with each treatment group totalling 100 workers. Seven flight mills were used simultaneously. After an hour, bumblebees were removed from the flight mill and placed into a 2ml Eppendorf tube. Bumblebees were

sacrificed in a -20°C freezer and thawed the following day for thorax width measurements to be taken using digital callipers (Mitutoyo Digimatic RS 600-880).

#### 4.2.3 Data analysis

The Akaike information criterion and maximum likelihood estimation analyses were performed using bespoke Fortran 77 code. All other statistical analyses were performed using 'R' programming software version 4.2.1 (185) and the 'lme4' (228) package. All figures were made using the ggplot2 package (186).

##### 4.2.4.1. Flight mill

A histogram was produced for each response variable to visualise data distribution. The data did not adhere to a normal distribution and was subsequently log transformed.

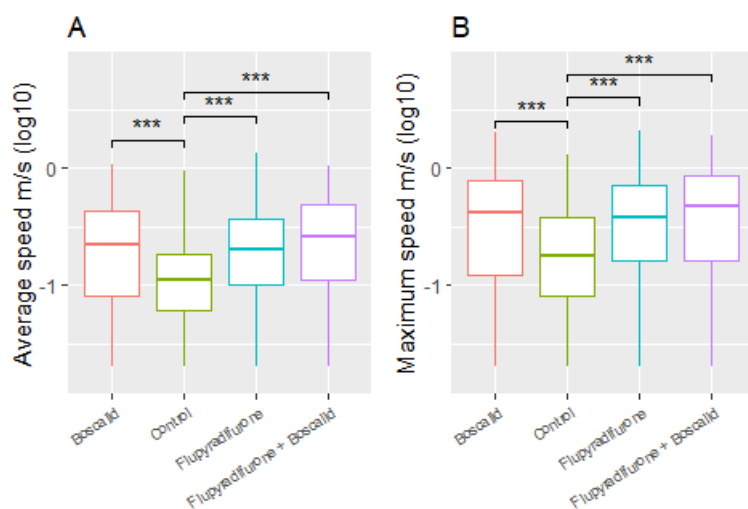
A linear model tested for any variation in mean thorax width between pesticide treatments with treatment as the only fixed effect. A TukeyHSD pairwise comparison test was conducted for each response variable (flight distance, flight duration, maximum speed, average speed, and pause duration) with pesticide treatment as the explanatory variable and colony as a covariate.

##### 4.2.4.2. Detection of power-law distributions

I tested for evidence of a pervasive 'idling activity' template in the flight patterns of bumblebees tethered to the rotational flight mill, following the approach of Reynolds *et al.* (2007) (230) as described in section 3.2.4.2.

##### 4.2.4.3 Propensity to fly

A generalized linear model was conducted in the same manner as described in section 3.2.4.3. As the random effect of colony did not account for any measurable variance in the data, it was eliminated to simplify the model and return it to a generalized linear model (GLM).



**Figure 4.3.1:** Boxplot of treatment versus log<sub>10</sub> average (A) and maximum flight speed (B). Data points represent individual flight bouts. The box edges represent the inter-quartile range (IQR), the horizontal line represents the median and the error bars represent 95-percentile range.

## 4.3 Results

### 4.3.1. Flight mill

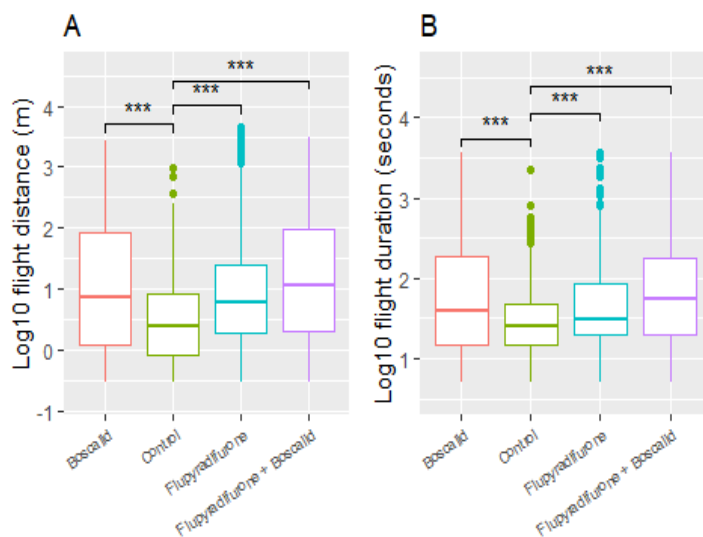
The results of the TukeyHSD pairwise comparisons revealed the average speed of bumblebees exposed to flupyradifurone (M=0.269 m/s, SE=0.240) and boscalid (M=0.287 m/s, SE=0.230) alone and in combination (M=0.327 m/s, SE=0.254) was significantly higher than bumblebees in the control group (M=0.154 m/s, SE=0.150) (Fig 4.3.1). This was true for maximum speed as well [flupyradifurone (M=0.496 m/s, SE=0.420), boscalid (M=0.487 m/s, SE=0.391), flupyradifurone and boscalid (M=0.564 m/s, SE=0.447), control (M=0.284 m/s, SE=0.270)]. Flight distance was also significantly longer for workers chronically exposed to flupyradifurone (M=225 m, SE=666) and boscalid (M=156 m, SE=397) alone and in combination (M=160 m, SE=427) compared with controls (M=15.8 m, SE=64.5) (Fig 4.3.2). This was also true for the flight duration [flupyradifurone (M=363 seconds, SE=920), boscalid (M=290 seconds, SE=682), flupyradifurone and boscalid (M=263 seconds, SE=598), control (M=52.3 seconds, SE=127)]. The pause duration between successive flights was significantly

longer in the boscalid group (M=971 seconds, SE=1450) compared with controls (M=471 seconds, SE=735). There was no significant difference in pause duration of the flupyradifurone, and flupyradifurone + boscalid group when compared with the control.

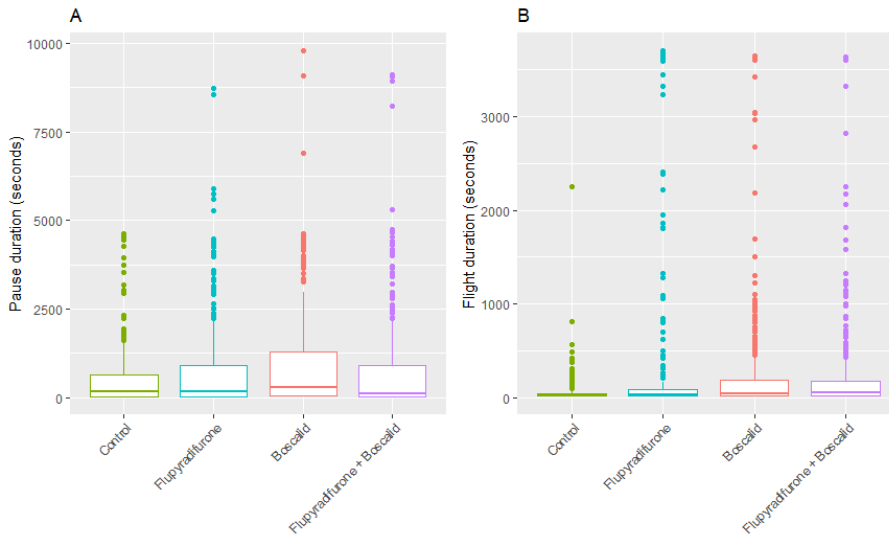
The linear model demonstrated a significant difference in thorax width between all groups (Table 4.3.2), with mean thorax width being lower in all treatment groups compared with the control. This could be due to a developmental effect of pesticide exposure. However, in the present study, the age of workers was not controlled or known when attached to the flight mill. Following a TukeyHSD pairwise comparison including colony as a covariate, it was found that one of the two colonies in each treatment group explained this significant difference in mean thorax width. *Ergo*, no significant difference of thorax width due to treatment.

#### 4.3.1 Power-law distribution

I demonstrate that there is very clear evidence of a power law in the flight duration and pause duration data. The Akaike weight (weight of evidence of the power law being the best model) is 1.00, indicating complete support for the power law. The maximum likelihood for the power law exponent (the power) is 1.53. A power law with exponent 1.65 for flight duration characterizes the control group, which is not significantly different from the treatment groups



**Figure 4.3.2:** Boxplot of treatment versus log<sub>10</sub> flight distance (A) and duration (B). Data points represent individual flight bouts. The box edges represent the inter-quartile range (IQR), the horizontal line represents the median and the error bars represent 95-percentile range. Outliers are plotted as filled coloured dots.



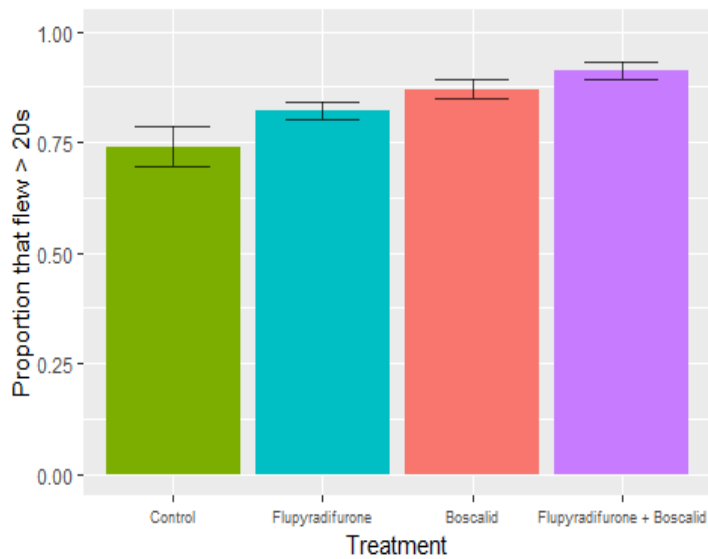
**Figure 4.3.3:** Boxplot showing the effect of treatment on flight (A) and pause duration (B) between successive flights. Data points represent individual flight bouts. The box edges represent the inter-quartile range (IQR), the horizontal line represents the median and the error bars represent 95-percentile range. Outliers are plotted as filled dots.

(boscalid 1.49, flupyradifurone 1.54, flupyradifurone and boscalid 1.44). The power law exponent for pause duration of the control group (1.40) was also not significantly different from the treatment groups (boscalid 1.35, flupyradifurone 1.36, flupyradifurone and boscalid 1.35). The largest departure from the expected  $3/2$  value for flight durations occurred for the combined treatment of flupyradifurone and boscalid. Bumblebees chronically exposed to flupyradifurone, boscalid, and both in combination, all behave normally in terms of flight and pause duration (Fig. 4.3.3).

#### 4.3.2 Propensity to fly

In total, 334 (83.5%) individuals initiated flight comprising 74% of controls (n=74 out of 100), 87% of boscalid (87 out of 100), 82% of flupyradifurone (82 out of 100), and 91% of flupyradifurone and boscalid combined individuals (91 out of 100) (Fig. 4.3.4). Bumblebees in the flupyradifurone and boscalid combined group had a significantly higher propensity to fly than the control group ( $Z=3.008$ ,  $p=0.003$ ) (Table 4.3.1). Flupyradifurone and boscalid had no

effect on bumblebee propensity to fly when exposed alone. Thorax width did not influence the propensity of individuals to fly.



**Figure 4.3.4:** Barplot showing the effect of treatment on the proportion of bumblebees that initiated flight ( $n=100$  per group). Error bars represent  $\pm$  SEM.

**Table 4.3.1:** Generalised linear model summary output for the effect of pesticide exposure (boscalid, flupyradifurone or boscalid + flupyradifurone) and worker thorax width on the propensity of an individual to initiate flight.

		Estimate	SE	z value	Pr(> z )
<b>Propensity to fly (n=400)</b>	Intercept	0.638	1.870	0.341	0.733
	Treatment - Flupyradifurone	0.507	0.384	1.320	0.187
	Treatment - Boscalid	0.888	0.405	2.195	0.028
	Treatment - Flupyradifurone + Boscalid	1.289	0.428	3.008	<b>0.003</b>
	Thorax width	0.079	0.359	0.220	0.826



**Table 4.3.2:** Linear model summary output for the difference of worker body size between treatment groups.

		Estimate	SE	t value	P value
<b>Mean thorax width (n=400)</b>	Intercept	5.174	0.038	134.789	<0.001
	Treatment - Flupyradifurone	-0.457	0.054	-8.412	<0.001
	Treatment - Boscalid	-0.422	0.054	-7.774	<0.001
	Treatment - Flupyradifurone + Boscalid	-0.267	0.054	-4.910	<0.001

## 4.4 Discussion

The present study, to my knowledge, is the first to assess the impacts of flupyradifurone and boscalid on bumblebee flight characteristics and the first to assess their combined effects on bumblebees. Chronic exposure to flupyradifurone and boscalid, alone and in combination, increased the average and maximum speed, distance, and duration flown by *B. terrestris* workers per flight. The power-law calculations demonstrate bumblebees behaved normally in terms of their pause and flight durations when exposed to flupyradifurone and boscalid, alone and together. Flupyradifurone and boscalid together increased the bumblebee's propensity to fly compared with controls but individually had no significant effect.

### 4.4.1. Flight mill

Bumblebees chronically exposed to flupyradifurone (4ppm) and boscalid (25ppb), alone and in combination, demonstrated an increase in their average and maximum flight speed per flight bout (Fig. 4.3.1). Average and maximum flight speed was highest in the combined treatment group, suggesting an interactive effect between flupyradifurone and boscalid in relation to flight speed. It is clear both of these agrochemicals are affecting the flight characteristics of bumblebee workers at conservative field-realistic doses. Flupyradifurone at very high doses (830ppm) impairs motor function in honeybees, decreasing their time spent walking and causing some individuals to walk in circles, lay upside down, and become immobile, however none of these behaviours were observed at the lower dosage of 83ppm (279). An increase in the average and maximum flight speed of bumblebees exposed to flupyradifurone cannot be explained by an impairment of motor function. It can, however, be explained by an excitatory effect, also observed in neonicotinoid exposure (197). Honeybees

acutely exposed to the neonicotinoid thiamethoxam experienced an increase in mean flight speed (240) and in bumblebees acute exposure to the neonicotinoid imidacloprid caused a significant increase to average speed (165). With neonicotinoids functioning by targeting the acetylcholine receptors in invertebrate nervous systems (241), they can acutely increase neuronal activity at field-realistic dosages (195, 196). The result of this may lead to hyperactivity. Flupyradifurone shares a similar mode of action with neonicotinoid pesticides due to their similar molecular structure (167, 280). As such, a potential explanation for the increased average and maximum flight speed we see in the present study could be due to an excitatory effect flupyradifurone causes similarly to neonicotinoids.

Boscalid has previously been shown to reduce the amount of adenosine triphosphate (ATP) present in honeybee flight muscles (281). ATP hydrolysis provides the energy for many essential processes in organisms and cells, including muscle contraction and relaxation (282). The mode of action of boscalid is to inhibit complex II in the electron transport chain, ergo preventing the production of ATP and halting fungi respiration (283). When in combination, flupyradifurone and boscalid appear to increase the flight speed of bumblebees, but a precise mechanism behind this observed affect is unknown. A reduction in the quantity of ATP in the flight muscles of bees combined with an increase in flight speed could result in the early onset of muscle fatigue and therefore, shortened flight distances or durations. However, that is not what I observe here.

I found flupyradifurone and boscalid increase the flight duration and flight distance of bumblebees when exposed alone and in combination (Fig. 4.3.2). Flight distance and duration was highest in the flupyradifurone treatment groups – suggesting an excitatory effect as previously discussed. Flight distance was shortest in the boscalid group (excluding controls) – signalling a potential link to the reduced quantity of ATP present in bee flight muscles (281) and therefore an early onset of fatigue and subsequent shorter flight distances. Flight duration was the shortest in the combined treatment group (when excluding controls) – suggesting an interactive effect. Previous studies on honeybees exposed to boscalid show a reduction of wingbeat frequencies in honeybees during flight, but did not alter the duration of flights (270) and could be explained by the reduction in ATP present in the flight muscles of honeybees (281).

#### 4.4.1 Power-law distribution

Bumblebees chronically exposed to flupyradifurone (4ppm) and boscalid (25ppb), behaved normally in relation to the duration of time spent flying and pause duration (Fig. 4.3.3). The power-law distributions of flight and pause durations of bumblebees in this experiment, from all groups, have an exponent close to  $3/2$ . This value indicates a null flight pattern; seen in healthy invertebrates flying in the absence of external stimuli (231). This null pattern is usually lost when animals are stressed (234-237). The findings of the present study suggest that *B. terrestris* workers' decision-making regarding the duration of pauses between flights and indeed the duration of flights, is unaffected by chronic exposure to flupyradifurone (4ppm) and boscalid (25ppb). My research provides further evidence to support the theory that "idling activity" is not a complex cognitive skill, which could be energetically expensive to maintain and potentially vulnerable to disruption from environmental stressors. Comparatively, it appears to remain robust in the presence of stressors, similar to what is seen in honeybees infected with the gut parasite *Nosema cerena* (146).

#### 4.4.2 Propensity to fly

Bumblebees chronically exposed to both flupyradifurone (4ppm) and boscalid (25ppb) for 2-weeks were more likely to initiate flight compared with the control group, but not when exposed to each agrochemical independently (Fig. 4.3.4). This observation provides further evidence for a potential interactive effect of these two agrochemicals on bumblebee flight behaviour. Pristine® is a common formulation containing two fungicides: boscalid (25.2%) and pyraclostrobin (12.8%). Pristine® significantly affects the learning performance of honeybees (284). Associative learning is a necessary ability for foraging, and several studies have demonstrated that exposure to agrochemicals can negatively affect navigation or foraging in bees, resulting in impaired learning (35, 80, 243, 260, 285). Therefore, there is concern that pristine® could impair foraging ability and weaken colony health. Moreover, when bumblebees are acutely exposed to the neonicotinoid imidacloprid, they make sub-optimal foraging decisions, increasing net energy expenditure (286). Combining these two examples with the findings presented in this chapter, it can be suggested that bumblebees chronically exposed to flupyradifurone and boscalid have an increased propensity to fly and potentially hindered foraging ability, which could ultimately result in weakened colony health.

Few studies have examined the sublethal effects of flupyradifurone and boscalid on wild bees (200, 201, 224, 287, 288). The small number that exist conclude that at field-realistic dosages, there are significant sublethal impacts to pollinators. With boscalid being the most commonly detected agrochemical in wild bumblebees (66) and flupyradifurone likely to replace neonicotinoids in areas with neonicotinoids resistance (221), there is a high chance that bumblebees will come in to contact with both of these agrochemicals, resulting in significant sublethal affects. I offer further evidence to support the idea that the existing form of the agrochemical regulatory process necessitates updating.

#### 4.4.3 Conclusion

Bumblebees are exposed to multiple types of agrochemicals in agricultural environments (66) and a number of these pesticides are referred to as 'bee-safe' at the dosages applied in the field. My results show that chronic exposure to conservative field-realistic doses of the neonicotinoid replacement, flupyradifurone, and one of the most commonly used fungicides, boscalid, have significant sublethal effects on bumblebee flight characteristics and behaviour. It is highly likely that the impacts of multiple chemical exposure on bees is being underestimated (33). Future work should aim to highlight the sublethal impacts of combined exposure of pesticides on key pollinator species, as I have here.

## **Chapter 5**

### General discussion

## 5. Chapter 5: General discussion

### 5.1. Thesis overview

In Chapter 1, I performed a systematic literature search highlighting advances made in the field of insect telemetry between 2012 and 2023. I found an upward trend in the number of insect telemetry papers published during this time; Apidae the most commonly studied insect family; harmonic radar to be the most commonly reported on tracking technology; and behaviour the most common study aim.

Within this chapter, I argue that the high number of studies using tracking technologies to investigate Apidae can be attributed to the fact that certain technologies were initially designed for bees and their design and functionality (e.g. RFID) are well-suited for studying central place foraging species, which includes many bee species. As a result, these technologies are commonly used in combination for studying bees and other central place foraging species. While there may be some truth in this statement, a more influential variable is the economic importance of these pollinators, as well as their positive public image (289). As a result, researchers are more likely to secure funding to work on them.

How can we address the species biases identified in Chapter 1? One option is to allocate research based on an economic value model, which is already reflected in my systematic map's findings. The species with the highest economic value, such as honeybees, tend to receive the most research attention. For example, honeybee pollination is estimated to be worth 12 billion USD annually in the USA alone (290). The geographical trends identified in Chapter 1 are intrinsically linked to the economic value of the study species. Another approach is to allocate research based on a conservation priority model, where resources are directed towards insect species suffering the greatest percentage decline. It is important to

acknowledge that funding influences the direction of invertebrate research and biases towards economically valuable species are likely. However, recognising the ecosystem value of insect species, in addition to economic value, is vital.

The capitalist sentiment of researching and conserving those organisms that provide the greatest economic worth is short sighted. Many invertebrate species play central roles in ecosystem services such as pollination (4, 291), herbivory and detritivory (292, 293), nutrient cycling (294, 295) and as food sources for birds, mammals, and amphibians. Without them, the stability of those ecosystems become threatened, with deleterious consequences (296-298). In the long term, the loss of keystone invertebrate species will cost the global economy far more than is needed to conserve them in the short term (9, 298).

A recent suggestion on how best to address insect declines states that experimentation in addition to quantitative analysis of existing data should be undertaken. The aim should be to identify the effect sizes for species-specific drivers to distinguish major from minor, alongside existing monitoring efforts (299). While the drivers in many cases have been identified, their relative significance has not been determined. The field of insect telemetry is well-positioned to aid in this endeavour and I concur with Weisser *et al*, 2023 that "...a coordinated effort is needed to produce the knowledge necessary for conservation action..." (300).

A key recommendation from the Kissling *et al.* (2014) review was to reduce the mass of electronic tags to limit the impact tag attachment has on normal organismal behaviour. I suggest, similarly to Kissling *et al.*, for each novel use of a technology on a species, the impact should be assessed as a priority and technologies should be further miniaturised where possible to reduce any affects to the organism. However, tracking techniques will always have limitations, even if they become lighter and smaller, there will be other constraints. For example, radio tags have become much lighter in the last few years (0.13g) (299) and future reductions in mass may provide percentage decreases but not by orders of magnitude. There is a limit to how small a technology can be while retaining functionality. Making tags more lightweight will begin constraining other factors such as tag lifetime and detection range. The tracking techniques, with their inherent properties, limitations, and boundary conditions could instead be properly implemented without need for continued technological advancements. For example, RFID tracking is ideal for quantifying foraging behaviour of

central place forager species such as the honeybee because the technique uses uniquely coded, light-weight tags combined with an automated detection system (95). It can never be used to track free-flying insects due to its short detection range, but to suggest the detection range should be increased through technological development so that it can, would be a waste of resources. Tracking technologies should instead be used in a way that makes the most of their strengths and circumvents their weaknesses. Although radio telemetry is the heaviest tracking technique in my review, it has been successfully utilised to track the normal movement behaviour of ground beetles (*Carabus ulrichii*) (301) and to study the effects of forest management on their movement behaviour (*Carabus olympiae*) (302). Using it on the heaviest, most robust insect family in my review reduces the weaknesses of the technology (i.e. relative large mass) and utilises its strength of long detection ranges (91).

Kissling *et al.* (2014) also recommended new and under-utilised technologies for insect telemetry, such as satellite-based tracking. Since their review, there has been an increase in the number of insect telemetry papers using GPS tracking. However, most of these tags are too large for direct attachment to flying insect species and so are used indirectly to map movement (303). Using GPS would provide advantages over other technologies (e.g. increasing the range capability of tracking migratory insects during migration). However, no satellite-based systems allow for this kind of data collection without the need for further investment in miniaturisation. As discussed in Chapter 1, the migratory patterns of monarch butterflies (*Danaus plexippus*) and common green darner dragonflies (*Anax junius*) across The Americas were tracked using continent-scale radio telemetry (133), proving that large scale tracking is possible with pre-existing infrastructure. These techniques may have downsides but they have and will continue to provide a powerful tool for disentangling enigmatic behaviours of invertebrate species.

I subsequently showed that despite technological advances since the Kissling *et al.* (2014) review, much can still be gained from more well-established techniques. An example of one such technology is the rotational tethered flight mill system. Using this apparatus combined with visual observations, I showed that common agrochemicals used in isolation and in combination (at field realistic doses) significantly affected flight and foraging behaviours of the buff-tailed bumblebee (*B. terrestris audax*).



In particular, in Chapter 2 I demonstrated bumblebee workers exposed to field realistic levels of sulfoxaflor flew significantly slower than those in the control group. I also found sulfoxaflor to reduce the distances flown and time spent flying of bumblebees compared with controls. When assessing the foraging activity of colonies exposed to sulfoxaflor (5ppb) and flupyradifurone (4ppm), alone and in combination, I found all treatment groups experienced an increase in activity compared with controls.

The increased level of foraging activity could be explained, in part, by the fact that sulfoxaflor and flupyradifurone target the same receptors in the invertebrate nervous system as neonicotinoid pesticides (166, 167). Neonicotinoid pesticides have been shown to cause an excitatory effect in honeybees (197). Honeybees acutely exposed to thiamethoxam experienced an increase in mean flight speed (240) and in bumblebees; acute exposure to imidacloprid caused a significant increase in average speed (165). Neonicotinoids can also acutely increase neuronal activity at field-realistic doses (195, 196). Exposure to these pesticides can result in hyperactivity. Because sulfoxaflor and flupyradifurone share their mode of action with neonicotinoids, they could be affecting bumblebees in the same way as neonicotinoids. This would explain the observations of increased foraging activity seen in Chapter 2. However, this excitatory phenomenon does not explain my flight mill data in Chapter 2, which showed a reduction in flight speed, duration and distance in bumblebees exposed to sulfoxaflor.

The explanation may be temporal. For example, in Chapters 2 and 3, I described the outcomes of studies in which bumblebees were attached to the flight mill apparatus for approximately 3 hours and 1 hour, respectively. Three hours may have been enough time for the bumblebees to experience muscle tiredness and early onset fatigue as seen in bumblebees attached to flight mills after exposure to the neonicotinoid imidacloprid (165). This would explain the decrease in flight speed, distance and duration of bumblebees exposed to sulfoxaflor in Chapter 2. It may be the case that during the time immediately after attachment to the flight mills, bumblebees display an increase in flight speed and distance, as seen in Chapter 3, but become fatigued due to the extra energy expenditure of performing these faster speeds over greater distances. Whereas, when quantifying the foraging activity of bumblebees in chapter 2 the maximum distance needed to fly to forage was 5 metres to the feeder and 5 metres

back to the nest. A very short and energetically cheap foraging trip, relative to the average foraging bout ( $442\text{m} \geq$ ) (123). As such, it would be unlikely to see any fatigue effects of the agrochemicals in this experiment and so we would only observe the hyperactive effects. This theory is further supported by a recent study demonstrating honeybees exposed to sulfoxaflor suffered a reduction in homing ability when individuals were displaced 1km from the nest (249).

From Chapter 2 and Chapter 3, I conclude that bumblebee workers chronically exposed to 5ppb sulfoxaflor for 2-weeks displayed an increase in foraging activity, flight speed (average and maximum) and distance flown over short time frames (1 hour). I also conclude that chronic sulfoxaflor exposure decreases the flight speed (average and maximum), distance, and duration of bumblebee flights over longer periods (3 hours). It is also important to note the sample size for treatment groups in Chapter 2 on the flight mills ( $n=25$ ) was smaller than in Chapter 3 ( $n=100$ ). I suggest future studies on the impact of chronic exposure of agrochemicals to bumblebee flight characteristics should have appropriate sample sizes and incorporate a temporal element in the experimental design to provide some clarity to the phenomena I describe here.

In Chapter 3, bumblebees were exposed to sulfoxaflor and flupyradifurone in the same way as in Chapter 2 and were attached to the flight mills for 1 hour. Bumblebees that come in to contact with these two agrochemicals fly faster and therefore further during their flights but do not fly for longer compared with untreated individuals. Bumblebees treated with both sulfoxaflor and flupyradifurone had the highest maximum flight speed of all groups, signalling a potential interactive affect between these two agrochemicals on flight characteristics of bumblebee workers. Interestingly, all the treated bumblebees behaved normally in terms of their flight and pause durations, as assessed by power-law distributions.

During movement behaviour, whether it be foraging, migrating or hunting, the time spent moving and the duration of subsequent pauses are an indicator of the organism's fitness and health (236). An injured animal, for example, would likely have a different looking distribution of movement and pause durations compared with a healthy, uninjured animal of the same species. As such, I would expect bumblebees exposed to a stressor that binds to receptors of the nervous system to display a different distribution of flight and pause durations compared

to a non-stressed bumblebee. In the absence of an external stressor, the time a bumblebee spends flying and remaining stationary should be power-law distributed over a range of scales of magnitude and characterised by exponents close to  $3/2$ . The control and treatment groups, in both Chapter 3 and Chapter 4, all had a power-law distribution of approximately  $3/2$ , suggesting that the agrochemicals, sulfoxaflor, flupyradifurone, and boscalid, at the dosages used here, do not affect pause and flight durations. These patterns have been observed in other species of bee as well as in a variety of moths and desert locusts, and appears to be a ubiquitous null movement pattern executed in the presence of minimal environmental stimuli (231). More complex movement patterns (i.e. not characterized by simple power-laws) are a hallmark of stressed individuals (236, 237, 304).

In Chapter 4, I showed that chronic exposure to field realistic doses of boscalid and flupyradifurone, alone and in combination, increased the average and maximum flight speed, distance and duration in bumblebees. I also showed that boscalid on its own, increased the pause duration between flights. These outcomes are similar to the effects seen in Chapter 3 except that boscalid and flupyradifurone increased the average flight duration where sulfoxaflor and flupyradifurone did not.

There is an apparent contradiction in the results of Chapter 4. When assessed by a TukeyHSD pairwise comparison, there is a significant difference in the flight and pause durations of bumblebees exposed to flupyradifurone and boscalid, and boscalid alone, but not when these parameters are assessed by their power-law distributions. This is because the TukeyHSD pairwise comparison identified the groups that were significantly different from one another. In this example, by computing the difference between each group mean and comparing the difference to the standard error of the estimate, the TukeyHSD pairwise comparison found a significant difference of flight and pause duration between the treatment groups and controls. Whereas, when analysing the power-law distributions, the goodness-of-fit of the power-law model shows that the overall shape (not range) of a distribution is characterized by a single number. This is the 'power law exponent' and is typically close to  $3/2$ . The shape of the distribution determines the relative proportions of long and short flight bouts but provides no information about the durations of the longest and shortest flights, hence the differing result from the TukeyHSD test.

I quantified the propensity of bumblebees to fly once attached to the flight mills in Chapter 3 and 4. Sulfoxaflor alone and in combination with flupyradifurone increased the likelihood of individuals to fly compared to the control. There is no sign of an interactive effect between these agrochemicals on propensity to fly, as the combined effect of these two pesticides is not significantly different from their individual effects. In Chapter 4, Boscalid combined with flupyradifurone was the only treatment to significantly affect this behavioural characteristic. From this, I demonstrate, at the applied dosages, sulfoxaflor affects the propensity of bumblebees to fly when exposed on its own and in combination with flupyradifurone. I also demonstrate that individually, boscalid and flupyradifurone, do not significantly affect the propensity of bumblebees to fly, but do when exposed together. This suggests that there is an interactive effect between these two agrochemicals on the likelihood of flight initiation in the bumblebee. The mechanism of which remains unknown.

As in Chapter 3, an increase in the likelihood of bumblebees to initiate flight combined with an increase in speed, distance travelled and/or duration spent flying will likely prove detrimental at the individual and colony level. Bumblebees exposed to any of the agrochemicals discussed here that leave the nest to forage may be disoriented (201), may become fatigued (section 4.4.1) and fail to return to the nest (249). With no apparent impact to the decision-making process in bumblebees (section 3.3.2 and 4.3.2), foragers may attempt to fly for longer periods and cover greater distances, than their non-stressed counterparts do, while still taking the same duration of pauses as non-stressed bumblebees. This may result in muscle fatigue and inefficient foraging, increasing the risk of not being able to return to the nest and ultimately lowering the colony's fitness and reproductive output.

## 5.2. Application of this work in the authorization process

For authorization of agrochemicals, the EU, USA, and many other countries require that risk assessments predict environmental exposure levels below concentrations deemed safe for non-target organisms (305). The majority of toxicological studies investigating the non-target effects of agrochemicals have concentrated on the western honeybee (*A. mellifera*), resulting in a substantial amount of evidence and detailed datasets available for evaluating the sublethal impacts on this species as part of risk assessments (306). However, wild pollinator species are important, but understudied and underappreciated (69, 306-309). Wild

pollinators are responsible for stabilising and increasing crop pollination services (20, 310); are able to pollinate at higher altitudes and lower temperatures than managed pollinators (311, 312); and wild bees have been shown to improve seed set, quality, shelf life, and market value of a variety of crops (23, 313-316).

However, datasets for wild bees (such as bumblebees and solitary bees) are incomplete and much smaller than for honeybees (306). From studies on wild bees, it has been found that there is a diverse range of sublethal effects in response to pesticide compounds across and within species (63, 317). Nevertheless, the results from tests on honeybees are applied non-discriminately to approximately 20,000 known species (Apoidea: Anthophila) (318) and do not take into account the impact of pesticides on other wild pollinators that are crucial for crop pollination (20).

Recreating the complex and diverse conditions of the natural world in a laboratory setting can be challenging. The environment is contaminated with numerous substances and stressors that can affect beneficial insects. When wild bees are exposed to a mixture of different types of stressors, the effects on them may be greater than those predicted from laboratory tests that expose them to only one stressor at a time (179). It has been argued that chemical risk assessments are flawed because they rely too heavily on laboratory toxicity tests, which do not accurately reflect the real-world conditions that reduce exposure and availability of agrochemicals (319). Despite these criticisms, laboratory experiments provide precise control over agrochemical concentrations and a more accurate evaluation of their effects. One possible explanation for these criticisms is that laboratory studies employ acute, short-term exposure routes that do not reflect the true field conditions. To gain a more accurate understanding of how non-target organisms are impacted by agrochemical exposure in the natural environment, the risk assessment process must closely resemble real-world conditions. Therefore, it is imperative that researchers continue to conduct chronic, multi-exposure studies, such as those discussed in this work, that evaluate the effects of field-realistic doses in combination with other stressors like nutritional stress, viruses, and parasites (33).

It is important to note that laboratory-based experiments are not the same as real-world field studies. To address some of the limitations of the pre-licensing process, Mommaerts and

Smagghe proposed a multi-tiered approach (64). This approach includes: (i) laboratory tests on individual bees, (ii) laboratory tests on key performance indicators such as worker survival and reproductive output, and (iii) semi-field trials to validate the laboratory results in a more realistic environment. This third step could be further modified to include multi-chemical exposure regimes at sublethal doses, taking into account the findings of this thesis.

### 5.3. Caveats and limitations

The pesticide chapters of this thesis involved treating only the feeding syrup (BioGluc) with the relevant pesticide, and not the pollen provided to the colonies. The reason behind this approach was the difficulty in diluting the pesticide in solid pollen pellets compared to liquid BioGluc. Nonetheless, this method does not reflect field realistic conditions, where bumblebees encounter agrochemicals in both nectar and pollen. Some studies have found that pesticide concentrations in pollen from treated plants are much higher than those in the nectar (156, 320, 321). This suggests that the data presented in this thesis may significantly underestimate the impact of these agrochemicals on wild bumblebees.

The flight mill is not a field-realistic proxy for free flight and therefore it has limitations regarding the extent to which results can be interpreted and extrapolated to the field (136). Tethered insects do not support their own body mass, which can lead to inaccurate and unnatural flight performances (322, 323). Additionally, while tethered, insects cannot control direction or turn, land, or generate lift due to the rigid nature of the flight mill arm. Furthermore, there are no weather conditions in general (see (324) for a review on flight mill limitations). A great number of studies have attempted to compare the speed between free flight and tethered flight (325-328) and have concluded that lower velocities are observed on flight mills.

Although, tethered flight mill data will not provide specific, field-accurate metrics *per se*, it will still provide comparative data. A comparative approach removes the many limitations listed, because all treatment groups in the experiment are subject to the same background variables. This approach quantifies the relative changes in behaviour, such as flight speed, duration, or distance, resulting from specific factors, such as age, temperature, or a stressor, rather than on the absolute values of these metrics.

A notable flaw of the flight mill is the minimal resistance of the rotating arm. As a result, when a bumblebee stops flying, it does not come to an abrupt stop. Instead, the arm continues to rotate and gradually slows. To address this limitation, it would be pertinent to exclude data 5-10 seconds preceding the end of a flight bout in future studies. However, it is important to note that other tracking techniques such as harmonic radar and radio telemetry also struggle with accurately identifying the end of a flight bout, as well as the beginning of the pre-landing and landing phases of flight when tracking free-flying insects. Therefore, despite these limitations, flight mills remain a valuable tool for studying insect flight performance.

#### 5.4. Future research directions

1. The focus should be shifted towards testing novel agrochemicals that are associated with detrimental impacts on bees. While there is already a large body of evidence against the use of well-established agrochemicals like neonicotinoids, it is important to reveal the consequences of the use of new chemicals on bees and understand their underlying mechanisms.
2. Prioritise research on non-*Apis* bee species in both laboratory and field settings. This recommendation is supported by the need for comprehensive investigations into the ecological impact of agrochemicals on a diverse range of bee species (152, 157).
3. The potential interactive effect of more than one agrochemical on pollinators is currently not considered during pre-licensing studies (unless they are part of a formulation); despite research demonstrating that bumblebees can be exposed to up to seven different pesticides simultaneously in agricultural environments (66). The work presented in this thesis, and published works (33), provides evidence that combination exposure of agrochemicals can be synergistic and is often overlooked.

#### 5.5. Final thoughts

The findings presented in this thesis highlight the sublethal effects that agrochemicals can have on wild bees at field realistic concentrations when used alone and in combination. By using flight mills and a foraging arena to assess the flight behaviours and capabilities of

bumblebees, I have demonstrated that these agrochemicals can have significant impacts on flight behaviour.

Given the crucial role of wild bees in maintaining crop yields (329), it is vital to adopt farming practices and agri-environment policies that balance proper pest management and yield with the protection of native pollinators and the environment. The ultimate goal should be to produce a system that benefits all parties. Taking steps to protect pollinators and their habitats will not only benefit farmers, but also contribute to the sustainability of our food systems and the conservation of vital ecosystems.

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## Supplement Chapter 1

### S1.1 Excluded categories from the literature search in WoS

- Acoustics
- Agricultural Economics Policy
- Agriculture Dairy Animal Science
- Agronomy
- Allergy
- Anatomy Morphology
- Andrology
- Anesthesiology
- Anthropology
- Archaeology
- Architecture
- Area Studies
- Art
- Asian Studies
- Astronomy Astrophysics
- Audiology Speech Language Pathology
- Automation Control Systems
- Biochemical Research Methods
- Biochemistry Molecular Biology
- Biotechnology Applied Microbiology
- Business
- Business Finance
- Cardiac Cardiovascular System



- Cell Biology
- Cell Tissue Engineering
- Chemistry Analytical
- Chemistry Applied
- Chemistry Inorganic Nuclear
- Chemistry Medicinal
- Chemistry Multidisciplinary
- Chemistry Organic
- Chemistry Physical
- Classics
- Clinical Neurology
- Communication
- Computer Science Artificial Intelligence
- Computer Science Cybernetics
- Computer Science Information Systems
- Computer Science Interdisciplinary Applications
- Computer Science Software Engineering
- Computer Science Theory Methods
- Construction Building Technology
- Criminology Penology
- Critical Care Medicine
- Crystallography
- Cultural Studies
- Dance
- Demography
- Dentistry Oral Surgery Medicine
- Dermatology
- Development Studies
- Developmental Biology
- Economics

- Education Educational Research
- Education Scientific Disciplines
- Education Special
- Electrochemistry
- Emergency Medical
- Endocrinology Metabolism
- Energy Fuels
- Engineering Aerospace
- Engineering Biomedical
- Engineering Chemical
- Engineering Civil
- Engineering Electrical Electronic
- Engineering Environmental
- Engineering Geological
- Engineering Industrial
- Engineering Manufacturing
- Engineering Marine
- Engineering Mechanical
- Engineering Multidisciplinary
- Engineering Ocean
- Engineering Petroleum
- Ergonomics
- Ethics
- Ethnic Studies
- Family Studies
- Film Radio Television
- Fisheries
- Folklore
- Food Science Technology
- Forestry

- Gastroenterology Hepatology
- Genetics Heredity
- Geochemistry Geophysics
- Geography
- Geography Physical
- Geology
- Geosciences Multidisciplinary
- Geriatrics Gerontology
- Gerontology
- Health Care Sciences Services
- Health Policy Services
- Hematology
- History
- History of Social Sciences
- History Philosophy of Science
- Hospitality Leisure Sport Tourism
- Humanities Multidisciplinary
- Imaging Science Photographic Science
- Immunology
- Industrial Relations Labor
- Infectious Diseases
- Information Science Library Science
- Instruments Instrumentation
- Integrative Complementary Medicine
- International Relations
- Language Linguistics
- Law
- Linguistics
- Literary Reviews
- Literary Theory Criticism

- Literature
- Literature African Australian Canadian
- Literature American
- Literature British Isles
- Literature German Dutch Scandinavian
- Literature Romance
- Literature Slavic
- Logic
- Management
- Materials Science Biomaterials
- Materials Science Ceramics
- Materials Science Characterization Testing
- Materials Science Coating Films
- Materials Science Composites
- Materials Science Multidisciplinary
- Materials Science Paper Wood
- Materials Science Textiles
- Mathematical Computational Biology
- Mathematics
- Mathematics Applied
- Mathematics Interdisciplinary Applications
- Mechanics
- Medical Ethics
- Medical Informatics
- Medical Laboratory Technology
- Medicinal Legal
- Medicine General Internal
- Medicine Research Experimental
- Medieval Renaissance Studies
- Metallurgy Metallurgical Engineering

- Meteorology Atmospheric Sciences
- Microbiology
- Microscopy
- Mineralogy
- Mining Mineral Processing
- Music
- Mycology
- Nanoscience Nanotechnology
- Neuroimaging
- Neurosciences
- Nuclear Science Technology
- Nursing
- Nutrition Dietetics
- Obstetrics Gynecology
- Oceanography
- Oncology
- Operations Research Management Science
- Ophthalmology
- Optics
- Ornithology
- Orthopedics
- Otorhinolaryngology
- Paleontology
- Pathology
- Pediatrics
- Peripheral Vascular Disease
- Pharmacology Pharmacy
- Philosophy
- Physics Applied
- Physics Atomic Molecular Chemical

- Physics Condensed Matter
- Physics Fluids Plasmas
- Physics Mathematical
- Physics Multidisciplinary
- Physics Nuclear
- Physics Particles Fields
- Physiology
- Plant Sciences
- Poetry
- Political Science
- Polymer Science
- Primary Health Care
- Psychiatry
- Psychology
- Psychology Applied
- Psychology Biological
- Psychology Clinical
- Psychology Developmental
- Psychology Educational
- Psychology Experimental
- Psychology Mathematical
- Psychology Multidisciplinary
- Psychology Psychoanalysis
- Psychology Social
- Public Administration
- Public Environmental Occupational Health
- Quantum Science Technology
- Radiology Nuclear Medicine Medical Imaging
- Regional Urban Planning
- Rehabilitation

- Religion
- Reproductive Biology
- Respiratory System
- Rheumatology
- Social Issues
- Social Sciences Biomedical
- Social Sciences Interdisciplinary
- Social Sciences Mathematical Methods
- Social Work
- Sociology
- Soil Science
- Spectroscopy
- Sport Sciences
- Statistics Probability
- Substance Abuse
- Surgery
- Telecommunications
- Theater
- Thermodynamics
- Transplantation
- Transportation
- Transportation Science Technology
- Tropical Medicine
- Urban Studies
- Urology Nephrology
- Veterinary Sciences
- Virology
- Water Resources
- Women S Studies

### **S1.2 Categorising study aims rules.**

#### **Foraging behaviour**

- Must include a measure of foraging ability or activity.

#### **Social behaviour**

- Must include a measure of social interaction

OR

- Must include a measure of social behaviour

#### **Navigation**

- Must include a measure of route learning

OR

- Must include a measure of route memory retention

OR

- Must include a measure of homing ability

#### **Dispersal**

- Must include an explicit measure of dispersal ability.

#### **Movement distance**

- Must include distance travelled by tagged insect.

#### **Habitat use and selection**

- Must include record of location

OR

- Must include habitat selection of insect.

#### **Impact of technique**

- Must include assessment of impact on tagged insect in comparison to untagged.

OR

- Must include assessment of attachment techniques in comparison to untagged e.g. glue type.



**Efficacy of technique**

- Must include first time use on tagged insect.

OR

- Must include detectability of technology in new environments.

OR

- Must include a comparison between techniques.

**Migration**

- Must include tracking of migration behaviour.