EFFECTS OF IMIDACLOPRID AND OCTOPAMINE ON THE HONEY BEE (*APIS MELLIFERA)* TROPHALLAXIS SOCIAL NETWORK

BY

SARAH MAGDALENA MURPHREE

THESIS

Submitted in partial fulfillment of the requirements
for the degree of Master of Science in Entomology
in the Graduate College of the
University of Illinois Urbana-Champaign, 2022

Urbana, Illinois

Master's Committee:

Professor Gene Robinson, Chair and Director of Research
Assistant Professor Adam Dolezal
Assistant Professor Marianne Alleyne

ABSTRACT

Honey bees (*Apis mellifera*) have been used as environmental sensors for chemical and nuclear compounds, either by tracking individual mortality, individual behaviors, or by tracking accumulated titers of toxins in individuals, honey, or wax. Tracking mortality does not capture sublethal effects, tracking individual behaviors (such as the proboscis extension response) are restricted to lab conditions, and tracking toxins requires time to build up concentrations and is often very intrusive or destructive. Instead, I was interested in using colony-level behavior as a sensitive and responsive biological sensor. In particular, the behaviors of foraging and trophallaxis – the exchange of fluid and information between bees, through which outside resources are spread rapidly – are attractive candidates because they link bees to the surrounding environment.

I used an automatic high-throughput monitoring system, called the "bCode" system, which involves bee barcode technology and machine learning-based behavioral detectors, to test whether changes in the trophallaxis social network can be tracked and used as an indicator of environmental toxins. In this study, all the individuals in paired colonies, composed entirely of one-day-old adult worker honey bees, were barcoded and placed into observation hives connected to an outdoor mesh enclosure, where they were provided sucrose solution at a feeder monitor, *ad libitum*, for 13 days. During the last four days, the colonies were treated (or not) via the sucrose solution at the feeder monitor. The treatments I selected were imidacloprid, a commonly used neonicotinoid pesticide, or octopamine, an endogenous biogenic amine.

By dividing the colony into task groups (either foragers or non-foragers) and calculating individual- and colony-level static network measurements, I found statistically significant

decreased movement and trophallaxis rates for non-foragers compared to foragers for both treatments, decreased movement rates for foragers treated with imidacloprid, and increased movement and trophallaxis rates for foragers treated with octopamine. In contrast, colony-level results showed no difference with either treatment. I speculate that these effects on individual behavior are consistent with the concept of "social immunity" to bolster colony health in the face of these "contaminants". In summary, while there are many factors that may not make the bCode system a viable option for a deployable biological sensor now, this study showed that it is a helpful tool to track a biologically relevant behavior that responds to environmental conditions.

ACKNOWLEDGMENTS

I would like to acknowledge the people that got me here - my mentors, my advisor Dr. Gene Robinson and my collaborator Dr. Tim Gernat, who patiently helped me grow as a researcher and a scientist; my lab members, especially Dr. Amy Cash-Ahmed and Nathan Beach, for teaching me the ins and outs of honey bee research; my committee members, Dr. Marianne Alleyne and Dr. Adam Dolezal, for providing feedback on my project and thesis; and my incredible friends, brothers, and parents for encouraging and supporting me.

TABLE OF CONTENTS

INTRODUCTION

Honey bees (*Apis mellifera*) have previously been used for environmental sensing, as they live in highly regulated societies that interact with their surrounding environment. Honey bees show age-related division of labor among worker bees, by which morphologically identical adult workers progress through different phases characterized by the performance of different duties (e.g., cleaning, nursing, guarding, etc.) (Free 1964). In typical colonies, after about two weeks of age, workers transition from jobs inside the hive to foraging outside of the hive (Winston 1991). This division of labor results in a specialized foraging force of bees that collect nectar, pollen, water, and plant resins from sources up to 6 km away (Visscher & Seeley 1982). With a foraging distance this large, bees likely encounter diverse pollutants on plants, either from inadvertent deposition or intentional application of pesticides (reviewed in Devillers & Pham-Delegue 2002). It is difficult to measure how these environmental contaminants manifest in honey bee colonies, which in turn can be a challenge when it comes to environmental sensing.

Previous research approached this topic by either tracking individual mortality, which will not capture sublethal effects, analyzing behaviors of individual bees, such as the proboscis extension response (PER) (reviewed in Devillers & Pham-Delegue 2002), or evaluating contaminants present in bee tissue, wax, or honey (reviewed in Bromenshenk et al. 2015). Although these methods are well established, the latter can be intrusive or destructive, relying on the build-up of contaminants. This build-up thus results in a delay between when the contaminant is detected and when it starts affecting the colony.

An alternative to monitoring individual behavior is to monitor the behavior of the whole colony. One advantage of this approach is that this type of monitoring exploits the collective

nature of honey bee life history, which can aid in amplifying an environmental signal depending on how the bees interact with each other.

Trophallaxis is an ideal behavior to monitor because it links bees to the surrounding area of a honey bee colony. Trophallaxis involves the constant exchange of fluid - a mixture of food, nucleic acids, and compounds related to neural and endocrine signaling (LeBoeuf et al. 2016). It is vital in intracolonial communication because outside resources are spread rapidly from the environment through a colony. Nixon and Ribbands (1952) found that a substance can spread throughout a colony in just a few hours by conducting experiments with radioactive sugar syrup, and although this shows that some of the substance is transferred between bees, not much is known about how much of or what substances are absorbed in bees during transit. Since trophallaxis has been suggested to be involved in pathogen spread (De Miranda & Genersch 2010; Ribiere, Olivier & Blanchard 2010; Lecocq et al. 2016) and communication (Winston 1991; Grüter & Farina 2009), it could be useful to track how environmental perturbations affect the trophallaxis network of a colony.

Shifts in individual behavior on a colony-level would be best captured using high-throughput automated behavioral surveillance systems. Benefits of these systems include collecting large amounts of data in an unobtrusive way with minimal daily manual upkeep. This automatic high spatiotemporal monitoring over extended periods of time has been utilized and improved upon for tracking ants (Mersch, Crespi & Keller 2013; Greenwald, Segre & Feinerman 2015) and bees (Wario et al. 2015; Crall et al. 2015; Gernat et al. 2018).

One of these technologies, called the “bCode system”, was first reported in Gernat et al. (2018) and subsequently used in other honey bee studies (Geffre et al. 2020; Jones et al. 2020; Gernat et al. 2021; Traniello et al. 2022). This high-throughput automated behavioral

surveillance system is made up of three parts: barcoded bees, automatic tracking of their movement, and behavioral detections. Briefly, images of barcoded bees in a colony are collected, and then used to identify barcodes, their locations, and their orientations in each frame using machine vision. With this information, the occurrences of different behaviors, such as worker egg-laying (Jones et al. 2020) or trophallaxis (Gernat et al. 2018), are identified via machine learning-based detectors and recorded.

To study whether there is a shift in colony-wide trophallaxis behavior in response to outside chemicals, my project exposed honey bee colonies to two substances: imidacloprid, a commonly used neonicotinoid pesticide, and octopamine, an endogenous biogenic amine. These were chosen because of their relevance to honey bee health and behavior, their differing modes of action, and the Robinson lab's previous experience with them (Wagener-Hulme et al., 1999, Schulz & Robinson, 1999, 2001, Schulz, Sullivan, and Robinson, 2002, Schulz et al. 2003, Fine et al. 2021).

Imidacloprid is a neurotoxic neonicotinoid insecticide commonly used as a seed treatment (Tomizawa & Casida 2005). In general, neonicotinoids affect insects by overstimulating their sensory systems. As such, imidacloprid is an agonist that binds to post-synaptic nicotinic acetylcholine receptors in the insect central nervous system, in which acetylcholine is a primary neurotransmitter (Sattelle & Breert 1990). Despite being seed treatments, neonicotinoids have been shown to contaminate soil and appear in local pollen that bees then encounter at sublethal doses and bring back to the hive (Krupke et al. 2012).

At sublethal doses, neonicotinoids have been shown to impair honey bee olfactory learning and memory (Decourtye et al. 2003; Aliouane et al. 2009; Williamson, Willis & Wright 2014), mobility, and communication (Medrzycki et al., 2003). Imidacloprid has also been found

to affect forager activity. Experiments using acute and chronic treatments of sublethal doses found that imidacloprid negatively influenced foraging performance (Cresswell 2011) and decreased forager activity (Decourtye et al. 2004). While the possible chronic sublethal effects of imidacloprid on whole colonies have not been studied, I anticipate that even at small doses, there will be a detectable difference. While imidacloprid is not a threat to humans, it represents a threat to pollinators and further, food security, and can serve as a model for future treatments because it has a rich literature.

Octopamine amine (OA) is endogenous to honey bees, integral for modulating behavioral processes in learning, memory, olfactory, visual, gustatory, and motor systems (reviewed in Barron & Robinson 2005). In addition, OA is a modulator of age-related division of labor among workers, especially the transition from working in the hive to foraging outside (Schulz & Robinson 2001; Schulz, Barron, and Robinson, 2002). OA levels in the antennal lobes are high at the onset of foraging (Schulz et al. 2003) and oral treatment of OA causes both direct and indirect effects on honey bee foraging. Direct effects on honey bees include an increase in foraging activity (Barron et al. 2005) and an earlier age at onset of foraging (Schulz & Robinson 2001).

OA also affects honey bee foraging indirectly, by affecting the "dance language" that is used for communication and recruitment (Seeley 1995). Dance duration communicates how far away a food source is and dance vigor communicates the quality of the food source (Visscher & Seeley 1982). When bees were treated with OA, dance vigor, duration, and likelihood increased with treatment (Barron et al. 2007), which may indirectly affect other bees' likelihood to forage. In contrast to non-treated bees, octopamine-treated foragers followed fewer dances (Linn et al.

2020), which may explain the increased likelihood of scouting behavior with treatment (Liang et al. 2012), a behavior responsible for exploring novel locations (Gilley 1998).

My project was composed of two experiments that monitored the trophallaxis social network with established automatic tracking methods (as used in Gernat et al. 2018). Over the course of two summers (June – September), colonies of barcoded honey bees were exposed to imidacloprid (in 2019) and octopamine (in 2020) at a sucrose solution feeder, located inside of a large mesh outdoor enclosure, where bees could fly freely. This method of treatment was a hybrid between oral and chronic methods because the treated sucrose solution was provided over multiple days, *ad libitum*, but only certain bees directly encountered it. This treatment method was chosen to mimic how bees would encounter substances naturally in their surroundings, while ensuring the bees were not encountering other food sources or any other contaminants.

Over the course of each 13-day trial, the hive and sucrose solution feeder were monitored with cameras that collected images and videos that were processed later for barcode and behavior detection. Since imidacloprid and octopamine are both known to affect foraging, my project utilized a feeder monitor, which unlike the hive monitor, was used for the first time with the bCode system to determine which bees had visited and when.

Using high-throughput tracking and machine-learning-based behavioral analyses, I tested (i) whether trophallaxis behavior changed with treatment and (ii) whether treatment differentially affected the foragers or non-forager behaviors. Based on previous work done with social insects, that predict that interactions among members of insect societies should be structured to slow down disease transmission (Geffre et al. 2020), I predicted that overall trophallaxis rates will decrease with treatment. I further predicted that the response to treatment will differ depending on task group (forager or non-forager). Since it is not known how much of these substances

(imidacloprid or OA) are transferred in trophallaxis, it is possible that the treatment will affect those directly in contact with the substance (foragers) rather than those that would acquire them indirectly (non-foragers). Finally, I also predicted that the response will differ depending on treatment (imidacloprid or OA) because they elicit different responses in honey bee behavior, as described above.

METHODS

EXPERIMENTAL DESIGN

I will refer to the experiment with the imidacloprid treatment as Experiment I, and the experiment with the octopamine treatment as Experiment II. This definition along with other differences between the experiments are summarized in Table 1.

Source colonies

Both experiments were conducted at the University of Illinois Bee Research Facility, Urbana, Illinois. Bees in this area are a mixture of European subspecies of *A. mellifera*, primarily *A. mellifera ligustica* and *A. mellifera carnica*.

Single-cohort-colonies (Robinson et al. 1989; Gernat et al. 2018; Geffre et al. 2020), composed entirely of one-day-old adult workers and a queen, were used throughout this study. To obtain the one-day-old adult workers, honeycomb frames with sealed pupae from source colonies derived from naturally mated queens, were removed and transferred in a Styrofoam box to an incubator. In the incubator, they were kept in emergence boxes at 34° C and 50% humidity. Bees were removed from frames daily to collect adults less than 24 hours old. When setting up a trial, bees were collected and barcoded within the same day.

Barcoded bees

One-day-old worker bees were cold-anesthetized and barcoded with custom unique "bCodes" as in Gernat et al. (2018). Barcodes were printed by Reliance Label Solutions, Inc. (Paola, KS) at a resolution of 800 dpi on Food and Drug Administration-approved 70# CIS Litho

paper (Avery Dennison Label and Packaging Materials) and protected with a Matte Self Wound Polypropylene laminate (QSPAC).

To apply barcodes, the bees were positioned using soft forceps (BioQuip) and a small amount of Loctite Super Glue Gel Control (Henkel) was applied to the center of the thorax. A barcode was placed using a wooden applicator, such that the orientation of the bCode was parallel to the length of the bee. Once the barcodes were attached, bees were placed in a plastic dish until they recovered from the anesthetization and the glue dried. They were then moved to a bin lined with Fluon®-coated walls (Insect-a-Slip, Bioquip), which was supplied with a piece of honey-filled comb to prevent starvation.

After bCode quality checks to ensure barcodes were attached securely and in the correct orientation to the center of the thorax, barcoded bees were carefully placed into a custom glass-walled observation hive containing a single one-sided plastic honeycomb frame, as in Gernat et al. (2018). A naturally mated queen, unrelated to the workers, was anesthetized with CO_2, barcoded, and transferred to the observation hive after they had recovered. Since Experiment II was conducted during the COVID-19 pandemic, safety precautions decreased the amount of field assistants that could share the same place to apply barcodes to bees. To ensure that the experiments could still be performed, the colony size was smaller than for Experiment I trials (Table 1).

Experimental timeline and conditions

Before the start of each trial, the frames were provisioned as follows: the top 12 rows of honeycomb cells were filled with honey (~0.1 ml per cell, ~75 ml total) and the following four

rows were filled with pollen paste (a 45:45:10 ratio by weight of honey, ground pollen, and water; ~0.1 ml per cell, 25 ml total;).

On the same day that the bees were barcoded, two provisioned observation hives were transferred to on-site sheds, which were kept at a temperature of 32.5 C and a humidity of 50%. Each hive was connected to the outside via a plastic entrance tube, and each colony was free to forage in a separate half of an outdoor mesh enclosure (6 m wide x 20 m long x 3 m high). To account for the differences in solar illumination between each half of the enclosure, I rotated control and treatment colonies between the north and south halves of the enclosures for each trial.

For the first two days of the experiments, the entrance tube was blocked with a piece of metal screen, to prevent the young bees from exiting the hive before their flight muscles properly developed (Roberts & Elekonich 2005). After sundown on the second day, the screen was removed and the bees were allowed to fly freely and eventually forage on the provided pollen and sucrose solution (described below) for the remainder of the experiment. Two monitors (described below) were set up to collect data about bee orientation and location: one for the hive interior and one at the sucrose solution feeder. For the next eight days, conditions were kept constant between the treatment and control colonies. Treatment of imidacloprid or octopamine started on day 10 and lasted for four days, and was administered at the sucrose solution feeder. After sundown on day 13, the hive entrance was blocked. The next day colonies were frozen to end the experiment and to recover the bees for census purposes (Table 1).

Daily maintenance

Pollen was ground and replaced daily, such that ~1/4 cup was available for the bees to feed *ad libitum.* Likewise, the sucrose solution feeder, containing 30% sucrose solution with a drop of pure orange blossom extract per 100 mL as an attractant, was cleaned and replaced daily. The amount of sucrose solution was different between the two experiments (Table 1) because bees did not consume the entire volume in Experiment I, so the volume was thus decreased for Experiment II.

Once a day, bee shed humidifiers and dehumidifiers were checked, and entrance monitor windows were cleaned and replaced. Twice a day, the hive windows were cleaned and replaced to avoid wax and nectar buildup, which would obscure areas of the hive from the monitor.

Experiment I

This experiment was comprised of six trials of paired treatment and control colonies, occurring between June to September 2019. These trials were performed by Tim Gernat and the analysis and synthesis of results were performed by me. Each colony had an initial size of 1,200 barcoded worker bees. Bees were provided with 400mL of sucrose solution each day to feed *ad libitum.*

During the last four days of the trial, the sucrose solution for treatment colonies was prepared with increasing concentrations of imidacloprid (Sigma, CAS: 138261-41-3) at the following doses: 1, 10, 25, and 50 µg/L. These doses were chosen because previous studies involving chronic sublethal treatments of imidacloprid found behavioral effects with doses ranging from 5 µg/L to 100 µg/L (Kirchner 1999, Decourtye et al. 2003, Decourtye et al. 2003, Faucon et al. 2005, Dively et al. 2015), with a reported high range of residues present in pollen

and nectar in seed treated crops ranging from 5 µg/L to 20 µg/L (Dively et al. 2015). The chosen doses extend below and above what would be encountered in field realistic conditions. Compared to the LC_{50} of imidacloprid for honey bees, 1760 µg/L (Cresswell 2011), the doses I used were substantially lower than lethal levels. Additionally, bees are exposed to variable pesticide residue from day to day and by including various doses in the experimental design, it reflected the inconsistent perturbations bees would encounter in the field.

At the end of each trial, colonies were closed and anesthetized with CO_2 for one minute before being immersed in liquid nitrogen, but colony counts were not performed. Even though they were not recorded, the final counts can be estimated from the bees' estimated time of death. Values for the final counts in Table 1 were retrieved from the detected barcode data and only bees that were alive on the final day of the trial were included in this calculation.

Experiment II

This experiment was comprised of six trials of paired treatment and control colonies, occurring between June to September 2020. These trials, along with the analysis and synthesis of results were performed by me. Each colony had an initial size of 800 barcoded worker bees. Bees were provided with 300 mL solution each day to feed *ad libitum.*

During the last four days of the trial, the bees from treatment colonies were exposed to sucrose solution that contained 2 mg/mL of (±)-octopamine hydrochloride (Sigma, CAS: 770-05-8) and 0.01M ascorbic acid (Sigma, CAS: 50-81-7). For control colonies, the sucrose solution was treated with 0.01M ascorbic acid (Sigma, CAS: 50-81-7). Previous studies treating bees orally have used doses ranging from 0.019 mg/mL to 1.9 mg/mL (Scheiner et al. 2002, Barron et al. 2007). I used a single dose in this experiment because OA studies, including those performed

in the Robinson lab, consistently used chronic treatments at a dose of 2 mg/mL (Schulz & Robinson 2001; Schulz et al. 2002; Barron & Robinson 2005; Barron et al. 2007).

At the end of each trial, colonies were closed and placed in a -40 freezer for 24 h. Colony counts were performed and these values were used for the final counts in Table 1.

BEHAVIORAL TRACKING

Hive monitoring

Barcoded bees were housed in a glass-walled observation hive with a one-sided plastic honeycomb frame, as in Gernat et al. (2018). The barcoded bees were unable to access the back side of the plastic honey comb, which meant that the images taken with the camera through the glass window could monitor all individuals at all times when they were inside of the hive (Fig. 1).

The hive was illuminated from the back and front by 850nm infrared light, which is not visible to bees (Menzel et al. 1988), and synchronized such that it was only illuminated during image capture. This was controlled by a CTL-IO-4 I/O Module (Smart Vision Lights). The images were captured at a rate of 2Hz with a 28.8 megapixel Prosilica GX6600 machine vision camera (Allied Vision) fitted with a Macro Apo-Componon 4.5/90 lens (Schneider Optics) with a broadband coating. These images were stored in a redundant array of independent disks (RAID). After the end of each experimental recording period, the images were copied onto a computing cluster (Biocluster, UIUC) for analysis. Images were captured, as described, from sunrise of the first day after set up (day one) to sunrise on day 14, for 24 hours a day, with the exception of unexpected camera outages (Fig. 2).

Feeder monitoring

Within the outdoor mesh enclosure, bees had access to pollen and sucrose solution to feed *ad libitum* (Fig. 3a). The sucrose solution was offered from a monitored feeder, which was comprised of three parts: the sucrose solution reservoir, the overflow container, and the camera (Fig. 3b). The reservoir contained the sucrose solution, and had a platform with a slit for bees to feed and access the sucrose solution. Any solution that escaped through the small hole in the side of this reservoir – used to regulate internal pressure – was collected in the overflow container below. The Raspberry Pi camera (8 megapixel v2.1, Adafruit) was affixed to an arm at the top of the reservoir, such that the feeding platform was in view of the camera, and captured video from 7:00 to 19:00 daily, with the exception of maintenance or unplanned outages (Fig. 2). A picture at the beginning of each hour was taken in addition, to enable checks for visual problems.

The Raspberry Pi camera was connected by a buried Ethernet cable to the main tracking computer housed in the bee shed (Fig. 1b). This cable also supplied power to the Raspberry Pi. The camera was controlled by a Raspberry Pi 2B computer running the Raspian 8 operating system. A custom shell script and the raspistill program were used to record videos of any bees collecting sucrose solution at the feeding slit. These videos were stored in the RAID array inside the bee shed. After the end of each experimental recording period, the videos were copied onto a computing cluster (Biocluster, UIUC) for analysis.

IMAGE PROCESSING

Barcode detection

Barcodes were detected in hive images as in Gernat et al. (2018). Barcodes from images obtained from the feeder monitors were also detected in this way, after the videos were first

converted into images using ffmpeg. The resulting data were filtered at each time step to remove bees with barcodes that were duplicated or associated with dead bees, using software developed by Gernat et al. (2018).

Time of death estimation

Time of death was estimated as in Gernat et al. (2018). Briefly, the time of death was the last time a bee was detected for at least four minutes during a five-minute window, when located above the bottom three rows of honeycomb, which is where dead bees tend to collect. This threshold was established because honey bees idle frequently in the hive. Lindhauer (1952) found that one bee idled on average approximately 78% of the night time hours and approximately 48% of the daytime hours. Furthermore, this is the standard for the bCode tracking technology, which has been verified with manual observations (Gernat et al. 2018).

Trophallaxis prediction

Once hive images were processed, analyzed, and filtered, trophallaxis identification was performed using a convolution neural network (CNN) as in Jones et al. (2020). Briefly, a trophallaxis event was flagged if an extended proboscis was detected between the heads of two bees. If trophallaxis was detected for an individual for over 3 seconds and under 3 minutes, it was considered to be the same event and the start time, stop time, and bCodes of the bees involved were recorded. Directionality was also identified and recorded; the “receiver” was the bee who extended her proboscis and mandibles, and the “donor” was the bee who only opened her mandibles.

Forager definition

In order to further divide the colony into the task groups of forager and non-forager, I used feeding visit information from the feeder monitor. These data were classified using a random forest machine learning algorithm trained on manually annotated videos of bees that collected sucrose solution at the monitored feeder, and bees that did not collect it to identify collection events. The resulting image classifications were then used as input to the Viterbi algorithm (Viterbi 1967) that uses a hidden Markov model (Baum 1972) to smooth over the classifications and thus identify the beginning and end of feeding events. The hidden Markov model captured the conditional probabilities that a bee remained in its current state (either collecting sucrose solution or not collecting sucrose solution) or switched states, and was parameterized using the same manually annotated data that was used to train the random forest algorithm. Feeding events of the same bee within 60 seconds of each other were grouped as part of the same feeding visit.

Despite the sharp drop-off in counts between bees that made one trip and those that made two (Fig. 4), I decided to use one trip as a cutoff, and defined a forager to be a bee that at any point in the experiment made at least one foraging trip. Using this criterion resulted in foraging forces that comprised between 5-15% of the colony (Fig. 5). In natural colonies, foragers make up about a third of the total population (Seeley 1986) and in smaller colonies, papers report a lower percentage, hovering around 16% (Robinson et al. 1989). Removing bees that only made one foraging trip during a trial would further decrease forager populations smaller than the expected foraging force in a small colony. Furthermore, once bees start to forage they continue to do so for the rest of their lives (Schulz, Barron & Robinson, 2002).

Movement tracking

The process of bCode detection produces information about an individual's location and orientation. Here, I only studied the change in location, i.e., how much a bee moved, which was calculated as in Geffre et al. (2020). Briefly, the rate was calculated as the total distance a bee moved for the treatment period divided by her time alive during that period.

DATA ANALYSIS

Static network analysis

To understand how trophallaxis behavior changed with treatment, the interactions and individual bCode identities involved over the course of the four-day treatment period were used to generate static networks for each colony. In this context, each bee is a node, each trophallaxis interaction is an edge, and the number of times two bees interact in the given time is the edge weight. These were generated using the igraph package in R.

Individual-level metrics

Static networks have properties that describe the qualities of the individual nodes, which I will hereafter refer to as "individual-level metrics" (e.g., how many partners a particular bee interacts with), as well as properties that describe the network as a whole, which I will hereafter refer to as "colony-level metrics" (e.g., how strongly the number of interaction partners varies among bees). For each individual, I calculated the node strength (number of interactions) and the node degree (number of interaction partners) across the entire duration of the treatment period, and then normalized these values by the length of time a bee was alive in the given period. This accounted for any bias the length of a bee's lifetime would cause in interaction activity.

Individual-level metrics were compared between individuals from control and treatment colonies. They were also compared between subgroups within these colonies: foragers vs. foragers and non-foragers vs. non-foragers. To compare these values, I used the lme4 package in R to run a generalized linear mixed model (GLMM) with trial as a random effect and colony (whether it was control or treatment) as a fixed effect. Despite the difference between experiments as to whether the dose increased or stayed the same during the treatment period, I performed an endpoint analysis looking at total values for my statistical comparisons. For this, I used a gamma distribution, as it had the lowest Akaike information criterion (AIC). The Benjamini-Hochberg correction was also used to account for multiple testing.

Colony-level metrics

I also calculated several colony-level metrics: number of connected components – the number of logical subdivisions within the whole network; edge density – the ratio of detected network edges to all potential network edges; mean node strength – the average number of interactions per bee; mean node degree – the average number of interaction partners per bee; assortativity coefficient – a measure of the preference a bee has to be connected to another bee with a similar number of interaction partners; clustering coefficient – the probability that two bees that share an interaction partner are also directly connected; degree distribution entropy – a measure of how strongly the number of interaction partners varies among bees. There are many other metrics to quantify static networks (Costa et al, 2007), I decided to use those colony-level metrics that were used in previous studies on network analysis in honey bee colonies (Gernat et al. 2018; Geffre et al. 2020).

Colony-level metrics were compared between control and treatment colonies. To determine whether a metric was significantly different between the two colony types, a Kruskal-Wallis test was used with a Benjamini-Hochberg p-value correction to account for multiple testing.

Movement analysis

To gather more information as to whether reduced interaction rates were driven by an overall reduction in activity, an analysis on bee movement was also performed. Using the output from the movement tracking processing, the rate or movement during the treatment period was calculated. To compare these values, I used the lme4 package in R to run a GLMM with trial as a random effect and colony (whether it was control or treatment) as a fixed effect. A gamma distribution was used to model the data, chosen as it had the lowest AIC tested. The Benjamini-Hochberg correction was also used to account for multiple testing.

Survival analysis

A survival analysis was performed using the survival and survminer packages in R. I used a univariate Cox proportional hazards (CPH) model with colony as a fixed effect to determine whether there were significant differences in mortality between control and treatment colonies, foragers, and non-foragers.

Software

Computer code for automatically locating and identifying bCodes in images, quantifying movement, and detecting trophallaxis is publicly available at https://github.com/gernat/btools.

For hive images, I used v0.13.1 of the barcode and trophallaxis detector and v0.15.0 of the movement detector, and for feeder monitor videos, I used v0.14.0 of the barcode detector. All statistical tests performed on processed data were performed in R version 4. 0. 3.

RESULTS

Mortality

In both experiments colony size declined at a steady rate over the course of the experiment, displaying high mortality during the day and low mortality during the night, with no differences between control and treatment colonies (Fig. 6; CPHs, P=0.355 for Experiment I; P=0.673 for Experiment II). This result indicated that there is no difference in mortality between control and treatment and thus likely did not affect my further findings for colony-level comparisons.

The survival curves for non-foragers were not different between control and treatment colonies (Fig. 7; CPHs, P=0.193 for Experiment I; P=0.491 for Experiment II). Survival curves also did not differ for foragers, either (Fig. 8; CPHs, P=0.078 for Experiment I; P=0.135 for Experiment II). As in the previous paragraph, these results indicate that mortality differences likely did not affect comparisons of forager interaction and movement patterns between treatment and control colonies.

Mobility

Bees tended to travel between 2,000-5,000 mm in an hour (Fig. 9). Movement rates decreased with treatment in both Experiment I (Fig. 9a; GLMM, $P < 0.001$, $\exp(\beta) < 0.001$) and Experiment II (Fig. 9b; GLMM, $P < 0.001$, $\exp(\beta) < 0.001$), although values between trials varied (Fig. 10). For control colonies in both Experiment I and Experiment II, the movement rates of foragers were lower than non-foragers (Fig. 11; GLMMs, $P < 0.001$, $\exp(\beta) < 0.001$ for Experiment I, $P < 0.001$, $\exp(\beta) < 0.001$ for Experiment II). This was also shown in treatment

colonies in Experiment I (Fig. 11a; GLMM, $P < 0.001$, $\exp(\beta) < 0.001$). There was no difference between forager and non-forager movement rates in treatment colonies in Experiment II, despite showing the same trend as the previous groups (Fig. 11b; GLMM, $P=0.12$, $\exp(\beta) < 0.001$). It is important to note that these data were normalized by the bees' lifetime, so forager movement rates were artificially decreased since they spend more time outside of the hive.

Within task groups, the effect of treatment depended on the type of treatment and the type of task group. Non-foragers had lower movement rates with treatment for both Experiment I (Fig. 12a; GLMM, $P < 0.001$, $\exp(\beta) < 0.001$) and Experiment II (Fig. 12b; GLMM, $P < 0.001$, $\exp(\beta) < 0.001$). While foragers in Experiment I had lower movement rates with treatment (Fig. 12a; GLMM, $P < 0.001$, $\exp(\beta) < 0.001$), while foragers in Experiment II had higher movement rates (Fig. 12b; GLMM, $P < 0.01$, $\exp(\beta) < 0.001$). This suggests that the exposure to imidacloprid decreased movement in both categories of bees, while exposure to octopamine decreased the movement of non-foragers, but increased the movement of those directly encountering the treatment.

Trophallaxis

Bees tended to engage in trophallaxis 1-3 times in an hour (Fig. 13). Trophallaxis rates per bee decreased with treatment in Experiment I (Fig. 13a; GLMM, $P < 0.004$, $\exp(\beta) = -0.006$) and remained the same in Experiment II (Fig. 13b; GLMM, $P < 0.225$, $\exp(\beta) = -0.003$), although values between trials varied (Fig. 14). I noted statistically significant effects but the means appear to be very similar. To explore if this is an artifact of the distributions, I looked at the medians.

Trophallaxis rates were higher in foragers than non-foragers for both control and treatment colonies across Experiment I and (Fig. 15a; GLMMs, $P < 0.001$, $\exp(\beta) = 0.061$ for control, $P < 0.001$, $\exp(\beta) = 0.068$ for treatment; Fig. 15b; GLMMs, $P < 0.001$, $\exp(\beta) = 0.069$ for control, $P < 0.001$, $\exp(\beta) = 0.105$ for treatment). This suggests that this difference was due to foraging status rather than a treatment-induced effect.

Within task groups, trophallaxis rates decreased with treatment for non-foragers in both Experiment I (Fig. 16a; GLMM, $P < 0.004$, $\exp(\beta) = -0.006$) and Experiment II (Fig. 16b; GLMM, $P < 0.04$, $\exp(\beta) = -0.005$). While foragers in Experiment I had no change in trophallaxis rates (Fig. 16a; GLMM, $P = 0.443$, $\exp(\beta) = 0.006$), foragers in Experiment II had higher trophallaxis rates (Fig. 16b; GLMM, $P < 0.009$, $\exp(\beta) = 0.032$). This suggests that only non-forager trophallaxis rates are affected by imidacloprid treatment in Experiment I, but that non-foragers and foragers have opposite changes in trophallaxis rates by octopamine treatment in Experiment II.

I also studied static trophallaxis network metrics and found no changes with treatment (Table 2), despite changes in the individual-level measures.

Foraging

The proportion of a colony's workers that became foragers did not differ with treatment of imidacloprid or octopamine (Fig. 5; Kruskal-Wallis tests, $P = 0.337$ for Experiment I; $P = 0.200$ for Experiment II). This metric may have shown some bias from the feeder monitor camera outages (Fig. 2), since the outages occurred when foragers were first detected. However, if a forager made multiple trips (which Fig. 4 illustrates is not an uncommon occurrence), it will still be recorded as a forager, just at a later time. For this analysis it was only important that a bee

was recorded at the feeder monitor at least once, it is unlikely that a camera outage missed a bee completely, however, it is still a possibility to consider.

DISCUSSION

In response to treatment, there were mixed findings with whether trophallaxis and movement trends tracked each other; bees in the forager group in Experiment I and bees in the whole colony in Experiment II had trophallaxis and movement trends that did not track, and in other groups, they did (Table 3). One explanation for the results reported here might simply be that if a bee moves more, she will encounter others more frequently and the opportunity to engage in trophallaxis is greater with the higher movement rate. On the other hand, the colony is densely packed with bees, so it should be easy to find a trophallaxis partner. Free (1956) has shown that bees will donate if hungry and receive if full, regardless of proximity, which suggests that more movement should not increase trophallaxis much.

In a study that used similar methods to mine, Geffre et al. (2020) found that trophallaxis rates decreased as a result of experimental infection with Israeli Acute Paralysis Virus (IAPV), but with an increase in movement. This further supports that reduced social interactions in my study were not driven solely by an overall reduction in activity. The range of values for trophallaxis and movement rates per bee in my study was consistent with Geffre et al. (2020), which used the same technology and same type of colony. They suggested that the treatment was likely only affecting the behavior of trophallaxis, instead of inhibiting a bee's opportunity to do so, and proposed that honey bees change their behavior to reduce social contact when the antiviral immune system of at least one participant is triggered (Geffre et al. 2020). This follows the ideas of social immunity that predict that interactions among members of insect societies should be structured to slow down disease transmission (Stroeymeyt, Casillas-Perez & Cremer 2014).

One difference between the Geffre et al. study and mine is that they treated colonies with a virus, while I treated colonies with a pesticide and a biogenic amine. This is important to note because this difference might also be reflective of alternative strategies of social immunity; viruses can multiply and increase with each trophallaxis interaction, while pesticide levels would dilute and diminish. With this in mind, I would thus expect an increase in trophallaxis rate to more quickly dilute the treatments I used in my study. Greenwald et al. (2019) found that in *Campnotus sanctus* ant colonies, foragers will unload only a portion of their crop load to several nestmates, who will drink from several foragers, and thus mix their crop content. This would dilute any potential poisons such that each crop contains fewer toxins, which might reduce death rate. I only found this increase in trophallaxis rates with the forager group in Experiment II, which may be better explained by octopamine's effect on honey bee behavior than by social immunity principles. My results for bees who directly encountered octopamine align with previous literature, citing that contact increased mobility (reviewed in Barron & Robinson 2005) and more vigorous dances, which would increase movement (Barron et al. 2007).

For non-foragers in both Experiment I and Experiment II, trophallaxis rates consistently decreased with treatment and mirrored the trophallaxis trends in response to a virus (Geffre et al. 2020). However, different task groups responded to treatments differently between Experiment I and Experiment II. In Experiment I, both foragers and non-foragers decreased movement rates with treatment. Assuming trophallaxis transferred some amount of imidacloprid from foragers to non-foragers, this aligns with the imidacloprid literature, reporting that contact exposure significantly decreased bee mobility (Lambin et al. 2001; Medrzycki et al. 2003) and queen mobility (Wu-Smart & Spivak 2016). In my study non-foragers had significantly lower trophallaxis rates, which aligns with another study that found this in response to individual

sublethal treatments of coumaphos, a type of organothiophosphate pesticide (Gregorc et al. 2018), although forager trophallaxis rates showed no significant difference.

While these results align with social immunity principles, it is also relevant to consider the results of my study from the perspective of how honey bees interact with xenobiotics. Honey bees have a long evolutionary history encountering and responding to different chemicals, and are, in some cases, attracted to chemicals that repel other insects, such as caffeine (Wright et al. 2013). I suggest that my results also are consistent with a social mechanism that responds to xenobiotics quickly. Considering that honey bees have a reduced number of immune genes (Evans et al. 2006), this could contribute to the hypothesis that they deal with xenobiotic threats in other ways – in this case, behaviorally. There are even differential behaviors, such as foragers collecting pyrethroid-contaminated pollen, that nurse and younger bees will not consume (Dolezal et al. 2015), that are suggested to be different mechanics to minimize contamination consumption in a colony. My results also show this differential response to an introduced xenobiotic in both Experiment I and Experiment II (Table 3). Studies show that bees prefer to forage on imidacloprid-contaminated food (Kessler et al. 2015), so Experiment I is a particularly good example of this because there was no change in trophallaxis rates for foragers, but within the colony the other bees responded differently, suggesting the colony is robust to these changes despite a preference for the contaminated food source.

In contrast to Experiment I, non-foragers and foragers in Experiment II had opposite responses to treatment in their movement and trophallaxis rates. As stated previously, those directly in contact with the treatment (foragers) exhibited mobility behaviors consistent with findings from the octopamine literature (reviewed in Barron & Robinson 2005). Those who received treatment indirectly (non-foragers), had reduced trophallaxis rates, which reflected the

reduction in social contact found in literature. Similar to honey bee non-foragers in Experiment II, Boulay et al. (2000) showed that octopamine reduced social contact in previously isolated carpenter ants, *Camponotus fellah*, suggesting that octopamine mediates behavior patterns linked to social bonding. In other words, relative to previously published findings, only foragers behaved consistently for movement, and only non-foragers behaved consistently for trophallaxis. It is not known how much treatment is transferred to other bees through trophallaxis, so this differential response to octopamine might be dose dependent, in that small doses are enough to reduce trophallaxis rates but high doses are required to reduce movement rates.

Despite having these differences in behavior between task groups and between experiments, Experiment I and Experiment II shared the trend of statistically significant decreased movement and trophallaxis rates in colonies as a whole. This could be due to either a larger effect size in one task group, which shifts the average toward the group with the larger magnitude, or it could be due to unequal group sizes, which shifts the average towards the majority's average. It is important to recognize that in the case of large sample sizes, effect sizes are more reflective of biological significance than p-values and bridge the gap between purely statistical significance and biological significance. It is the case that many of the effect sizes in my study appear to be quite small, suggesting the statistical significance could be strongly influenced by the very large sample sizes. However, what I defined to be foragers only made up 5-15% of the colony populations, so I speculate that the latter possibility is more likely, especially since whole colony results followed those of the non-foragers.

In contrast to the individual-level results discussed above, the colony-level results for both Experiment I and Experiment II showed no significant change with treatment. For both imidacloprid and octopamine treatment, there were no colony-level changes for the chosen

colony-level measures. One possible explanation is that the interactions between bees dampened the environmental signal. In colonies, these can be amplified or dampened depending on how the bees interact with each other, so I speculate that the environmental signal is amplified through their individual behaviors and dampened such that the overall network structure is not changed. A second possible explanation is that the sample size for the colony-level analyses was much smaller than the sample size for the individual-level behaviors. Analyses of colony-level behaviors are limited by the number of trials that can be performed. This is important because a large sample size can better detect small shifts, and in the context of large groups, any small reduction in movement or social contact can create a meaningful reduction in the spread of disease. While it is possible that my results are an artifact of large sample sizes, it is unlikely because I still found no differences in trophallaxis rates in Experiment I foragers and Experiment II whole colonies (Table 3). A third possibility is there are dozens of possible colony-level network measurements (Costa et al., 2007), so it is also possible that there are other measures that would have significantly changed that I did not choose to study.

There are other factors to consider that affected my analyses, the first of which is the bias introduced by how I normalized individual measures – by normalizing by the time a bee was alive during the trial. For movement rates, this normalization likely resulted in values for foragers that were artificially lower than their actual movement rate. This was because foragers spend more time away from the hive than non-foragers, making one or more foraging trips, which will record as no movement to the hive monitor for portions of the day, and thus decrease calculated movement rate. A more accurate calculation for the movement rate would be to normalize by actual time spent on the frame instead.

Trophallaxis rate was not as strongly affected by the normalization I used as movement rate was for a couple of reasons. First, a bee could reasonably travel 2,000 mm in an hour (Fig. 9) but in that time, only engage in trophallaxis twice (Fig. 13), so any time away from the hive monitor will affect movement rate more than trophallaxis rate. Furthermore, foragers tend to not engage in trophallaxis outside of the hive, so most interactions would be captured by the hive monitor. In other words, if foragers had a decreased trophallaxis rate, it was more likely because of the treatment itself and not influenced by data analysis technique.

Another bias arising from these experiments was the occasional occurrence of feeder monitor camera failures in treatment colonies (Fig. 2). While this did not strongly affect the analyses presented here, it prevented me from comparing foraging rates between control and treatment colonies. The other analyses only depended on whether a bee was detected at the feeder monitor (to be defined as a forager), while foraging rate depended on the number of times a bee was detected at the feeder monitor. One solution to this would be to simulate outages experienced by one colony in the paired colony at levels actually experienced by the other colony – this would make the treatment and control data comparable and would take into account the varying foraging rates throughout the day, with less activity in the morning and more in the afternoon (Pernal & Curie 2001; Ghosh et al. 2020). For example, an outage in the afternoon would likely miss more detections of bee visits and affect foraging analyses more than an outage in the morning. The process to make this type of adjustment was too involved for the scope of my thesis.

Considering the results presented here, is it feasible to use this high-throughput automated behavioral surveillance system in the field as a real-time bioindicator? There are a couple of disadvantages that might prevent this goal. The monitoring system setup itself has

many limiting factors: it requires expensive specialized equipment, climate-controlled sheds in the field, electrical power accessibility, and creating single-cohort-colonies of barcoded bees is extremely labor intensive. A real-time analysis is hindered by the image processing time and also the fact that classifications of bees into task groups are retroactively applied. After the analyses are completed and the definitions applied, this setup then requires multiple trials in order to gather enough data for statistically significant results and control colonies in order to make comparisons and detect changes. Colonies, and by extension the trophallaxis network, vary due to intrinsic (see Fig. 10 and Fig. 14) and extrinsic factors (reviewed in Crailsheim 1998). This means a global trophallaxis or movement rate cutoff, below which indicates a known contaminant in the colony, cannot be applied and suggests that these values are relative. Intracolonial comparisons (movement or trophallaxis rates of non-foragers to foragers) might thus provide a more powerful way to use honey bees as environmental sensors. My results show that non-forager and forager behavior can shift in different ways, which means that values from one task group could be compared to values from the other task group without the need for a control colony.

With these considerations in mind, I still consider this study a successful proof-of-concept – this behavioral monitoring system detected an effect of environmental "contamination" on the honey bee trophallaxis network. While the bCode system may not be a viable option to be used as a bioindicator now, it is a helpful tool to track a biologically relevant behavior that responds to realistic environmental conditions, such as imidacloprid levels, which is reflected by numerous studies (Gernat et al. 2018; Geffre et al. 2020; Jones et al. 2020; Gernat et al. 2021; Traniello et al. 2022) and the present work. One of the possible extensions of this technology would be to detect contaminants that are present in the environment persistently,

rather than detecting a sudden increase in one contaminant. Imidacloprid is an example of a ubiquitous substance that bees encounter and with more studies, we could glean whether trophallaxis rates in colonies near high contamination areas are consistently lower.

Moving forward, this experimental setup could be used to test substances that affect both insects and humans, such as selenium, a prevalent soil-borne metalloid pollutant that has been found to negatively impact bumble bee and honey bee colony health (Hladun et al. 2012, Sivakoff et al. 2020). In contaminated natural areas, it has been found in pollen, propolis, honey from honey bee hives in close proximity to these areas (Bogdanov 2006; Bibi et al. 2008). Selenium contamination affects soils and plants in areas where insect pollination is a critical element to agricultural and natural ecosystems, and is also considered, by a screening assessment by Environment and Climate Change Canada (ECCC) and Health Canada, a direct risk to human health (ECCC and Health Canada 2017). Honey bees and honey bee products have already been used to assess heavy metal contamination (Jones 1987; Leita et al. 1996), which makes this a great candidate for a future application for the bCode technology.

As suggested by results from other studies, a reduction in trophallaxis due to exposure to pathogens or pesticides might serve as an adaptive social immunity mechanism or response to xenobiotics, which by reducing social contact between individuals, will reduce transmission of harmful substances (Cremer, Pull & Fürst 2018; Stroeymeyt et al. 2018). While these social immunity studies apply to pathogens, my experiments showed that this phenomenon occurred in pesticide and biogenic amine treatment as well, suggesting a universal method of mitigating environmental stressors in honey bee colonies.

TABLES AND FIGURES

Table 1 – Differences between experiments. Factors not listed are assumed to be the same (i.e., length of trial, length of treatment, amount of pollen provided, daily maintenance procedures, etc.).

	Experiment I	***Experiment II***
Year	2019	2020
Treatment	Imidacloprid	Octopamine
Initial colony size	1,200 workers	800 workers
Final counts	601 ± 183 bees	561 ± 134 bees
Dose concentration	Increasing	Constant
Feeder volume	400 mL	300 mL

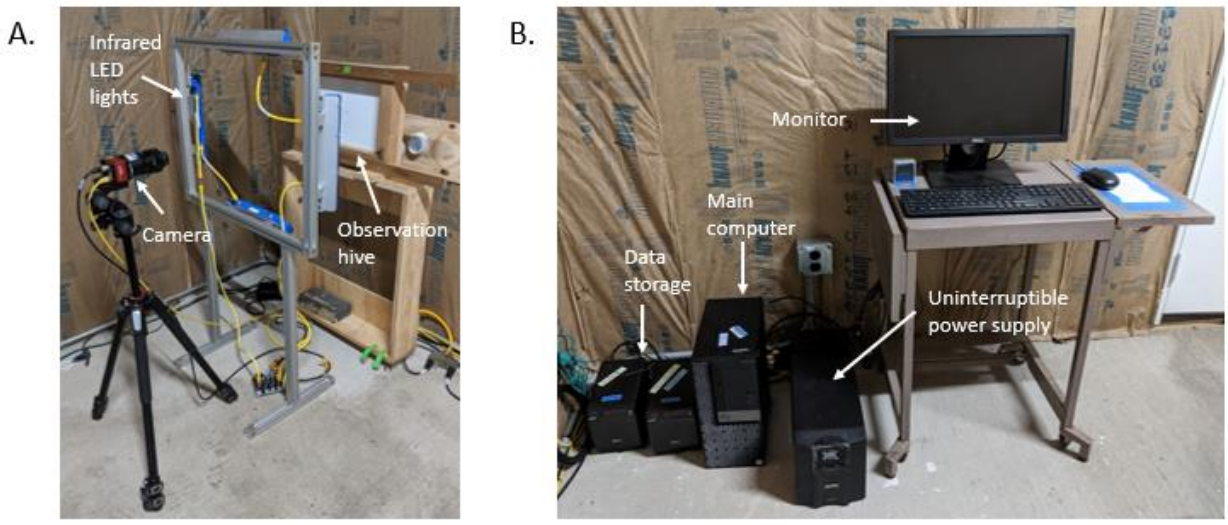

Figure 1 – Interior bCode tracking set-up: **(A)** Tracking rig (two per shed), consisting of an observation hive that housed a colony of barcoded bees, a set of infrared LED lights that illuminate the observation hive invisibly to the bees, and a camera that captures images at a rate of 2 Hz. **(B)** Computer system (one per shed) for operating two bee tracking rigs. This system also controls the computers that monitor the sucrose solution feeders in the outdoor mesh enclosure to which the observation hives are connected, and stores their video feeds.

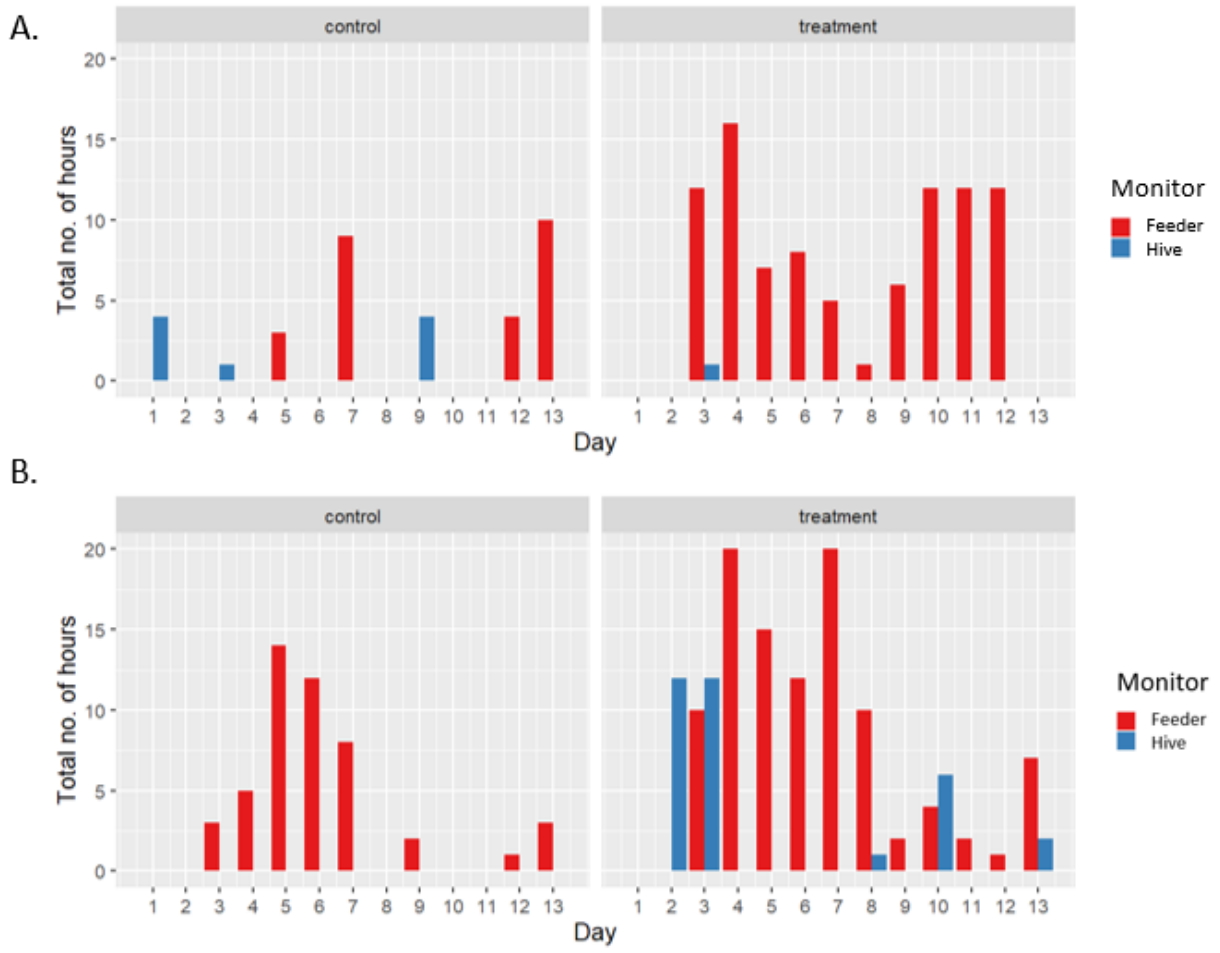

Figure 2 – Plot of camera outages over each trial day for the feeder and hive monitor cameras, summed over the six trials for (A) Experiment I and (B) Experiment II. In both experiments, for unknown reasons there were more feeder monitor camera outages in the treatment colonies. A note on the hours of outages – since this shows the cumulation of six trials, the maximum time a feeder monitor will record in a day over each trial is 6*12hr = 72hr, and the maximum time a hive monitor will record in a day over each trial is 6*24hr = 144hr.

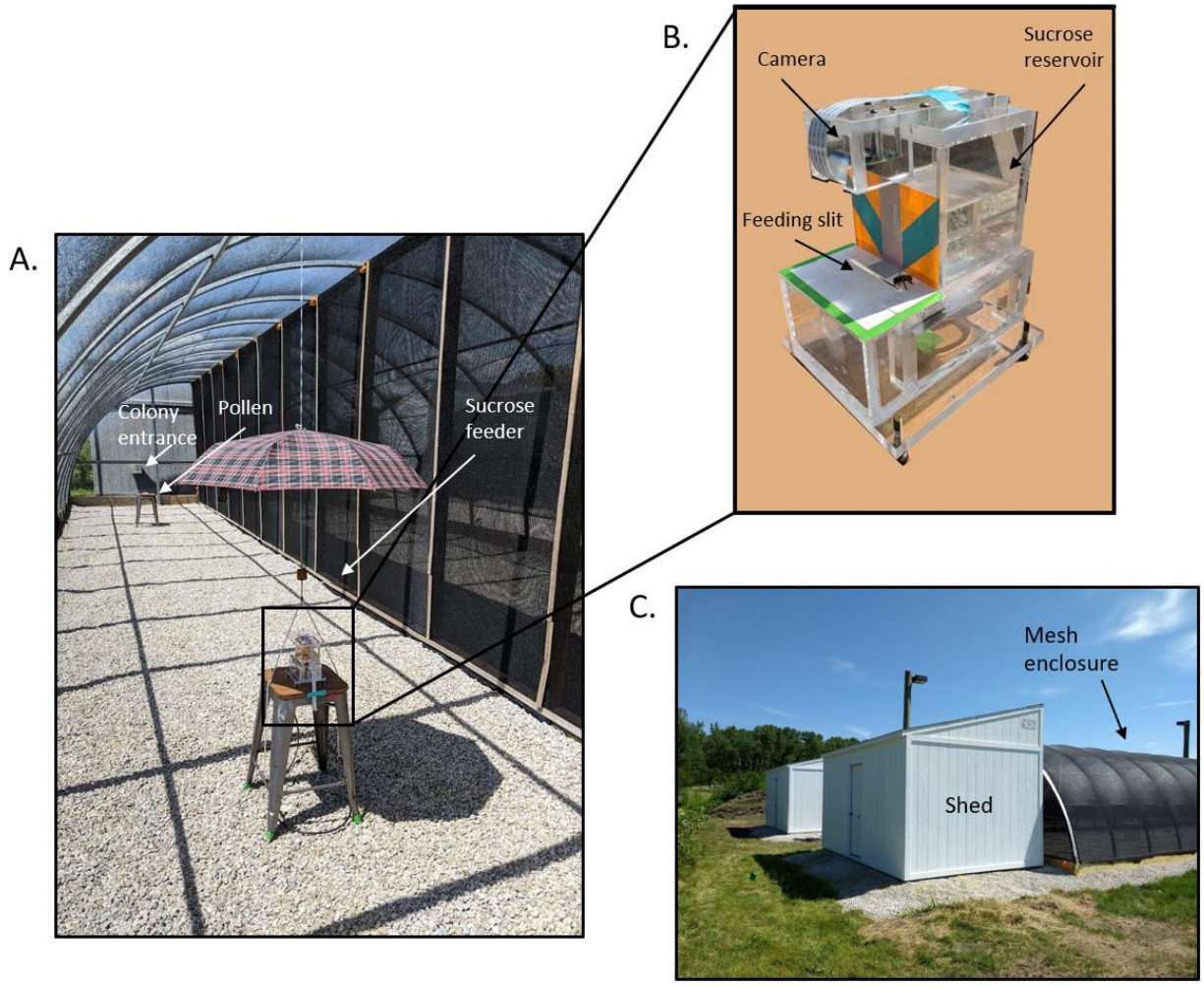

Figure 3 – Exterior bCode tracking set-up: **(A)** Inside of the mesh enclosure, where bees exit the colony entrance to access provided pollen and sucrose solution. This mesh enclosure is adjacent to another with the same setup. **(B)** Feeder monitor for detecting bees feeding on the sucrose solution. It consists of a camera, reservoir, and a feeding slit for bees to collect the solution. The camera is connected to a Raspberry Pi computer that records video at 10 Hz and communicates with the IT station inside of the shed. (C) Outside view of connection between the shed and mesh enclosure.

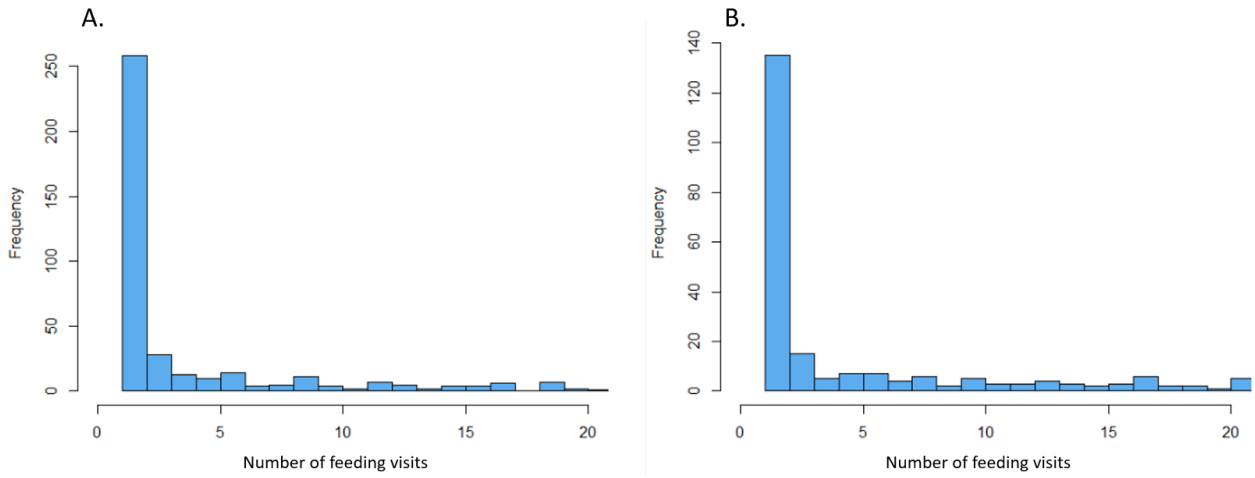

Figure 4 – Histograms of the pooled number of feeding visits to the feeder monitor per bee over the entire trial period (x-axis extends past 20 feeding visits – condensed for clarity) across trials for (A) Experiment I and (B) Experiment II.

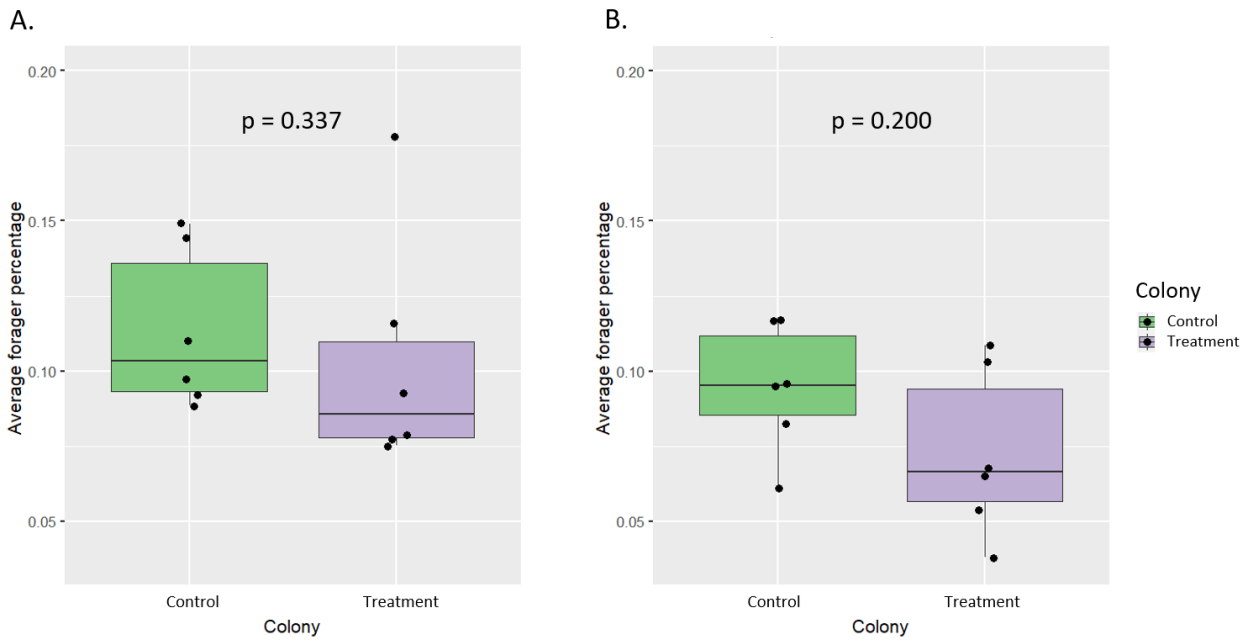

Figure 5 – Proportion of foragers in control and treatment colonies by the end of the trials (lower and upper hinges correspond to the 25th and 75th percentiles; whiskers extend to values 1.5*IQR outside of the hinges; line indicates median; points represent individual trial values) for (A) Experiment I (P = 0.337; n = 6 for control, n = 6 for treatment) and (B) Experiment II (P = 0.200; n = 6 for control, n = 6 for treatment).

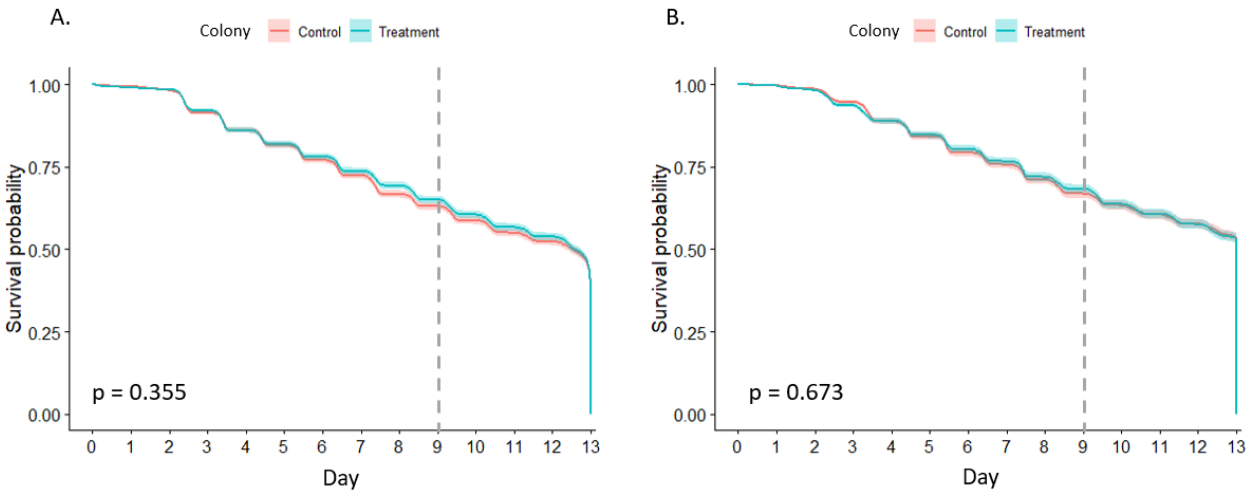

Figure 6 – Survival curves for the treatment and control colonies (shading represents 95% confidence interval; dotted grey line indicates the start of treatment) for (A) Experiment I (P = 0.355; n = 6795 for control, n = 6833 for treatment) and (B) Experiment II (P = 0.673; n = 4620 for control, n = 4641 for treatment). n.s.: no statistically significant effect of treatment with p > 0.05.

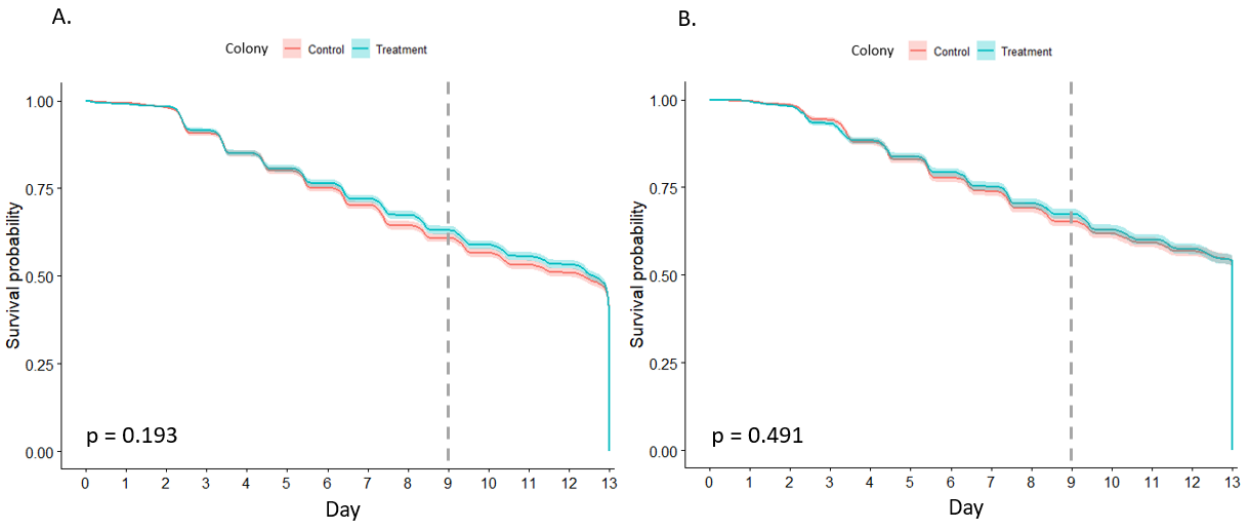

Figure 7 – Survival curves for the non-foragers of treatment and control colonies (survival plot as in Fig. 6) for (A) Experiment I (P = 0.193; n = 6252 for control, n = 6340 for treatment) and (B) Experiment II (P = 0.491; n = 4285 for control, n = 4371 for treatment). n.s.: no statistically significant effect of treatment with $p > 0.05$.

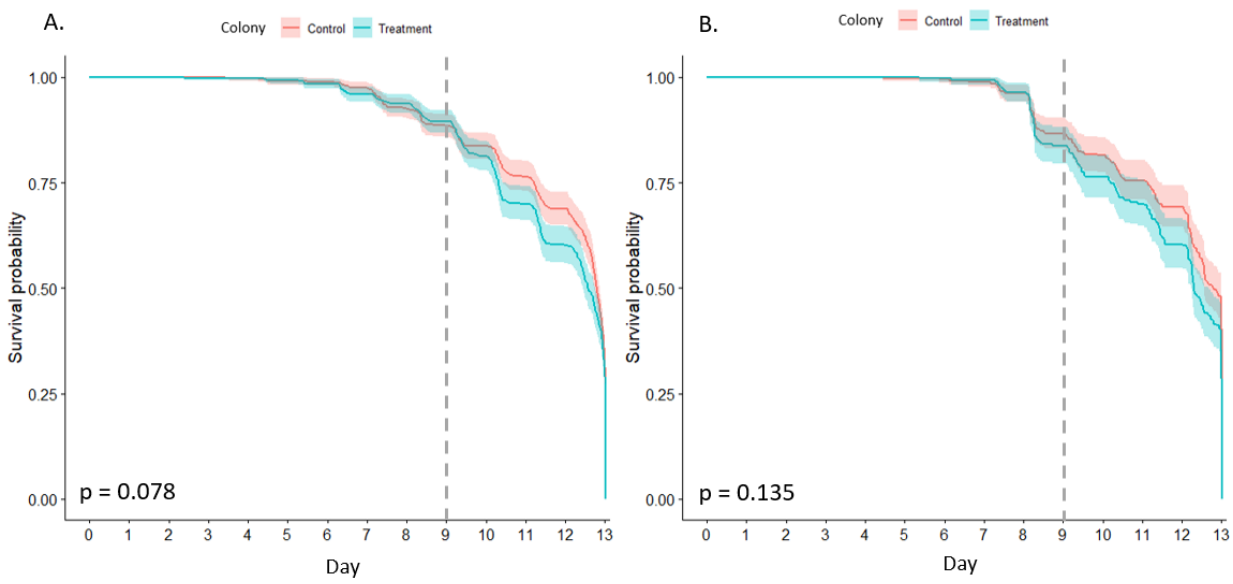

Figure 8 – Survival curves for the foragers of treatment and control colonies (survival plot as in Fig. 6) for (A) Experiment I (P = 0.078; n = 543 for control, n = 493 for treatment) and (B) Experiment II (P = 0.135; n = 335 for control, n = 270 for treatment). n.s.: no statistically significant effect of treatment with p > 0.05.

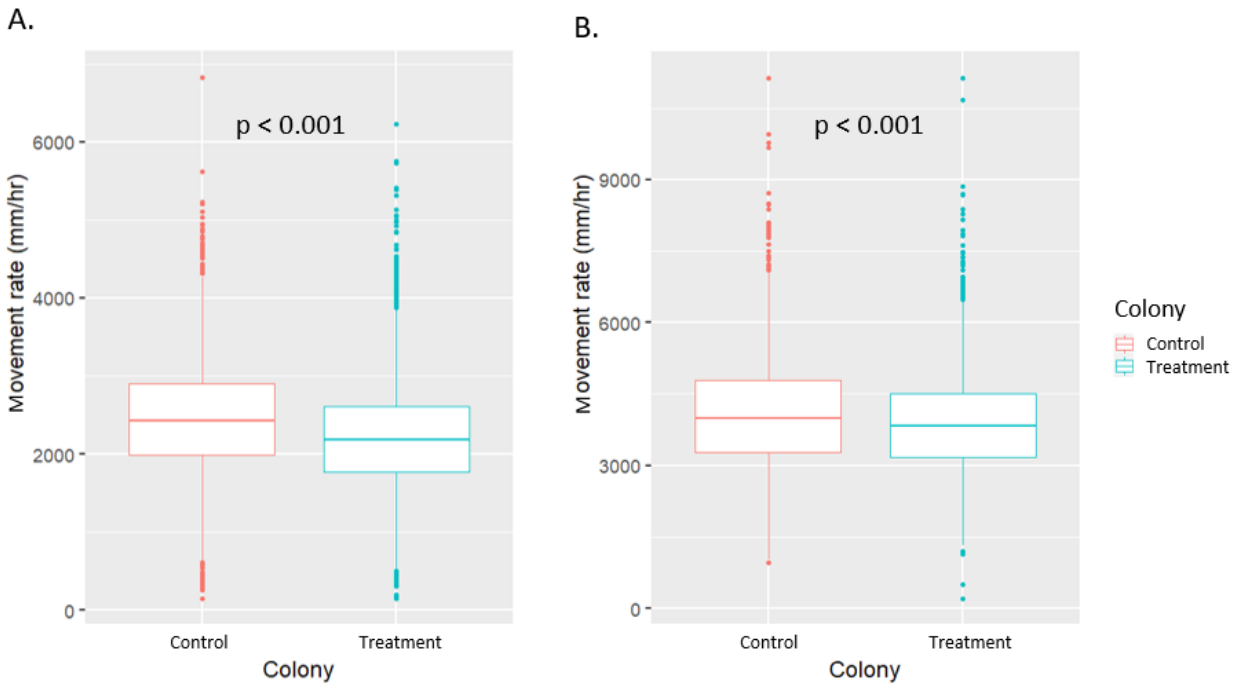

Figure 9 – Box plots of the normalized movement rates for bees in control and treatment colonies over the entire treatment period (lower and upper hinges correspond to the 25th and 75th percentiles; whiskers extend to values 1.5*IQR outside of the hinges; line indicates median; points represent outliers) for (A) Experiment I ($P < 0.001$; $\exp(\beta) < 0.001$; n = 4284 for control, n = 4436 for treatment) and (B) Experiment II ($P < 0.001$; $\exp(\beta) < 0.001$; n = 3086 for control, n = 3170 for treatment).

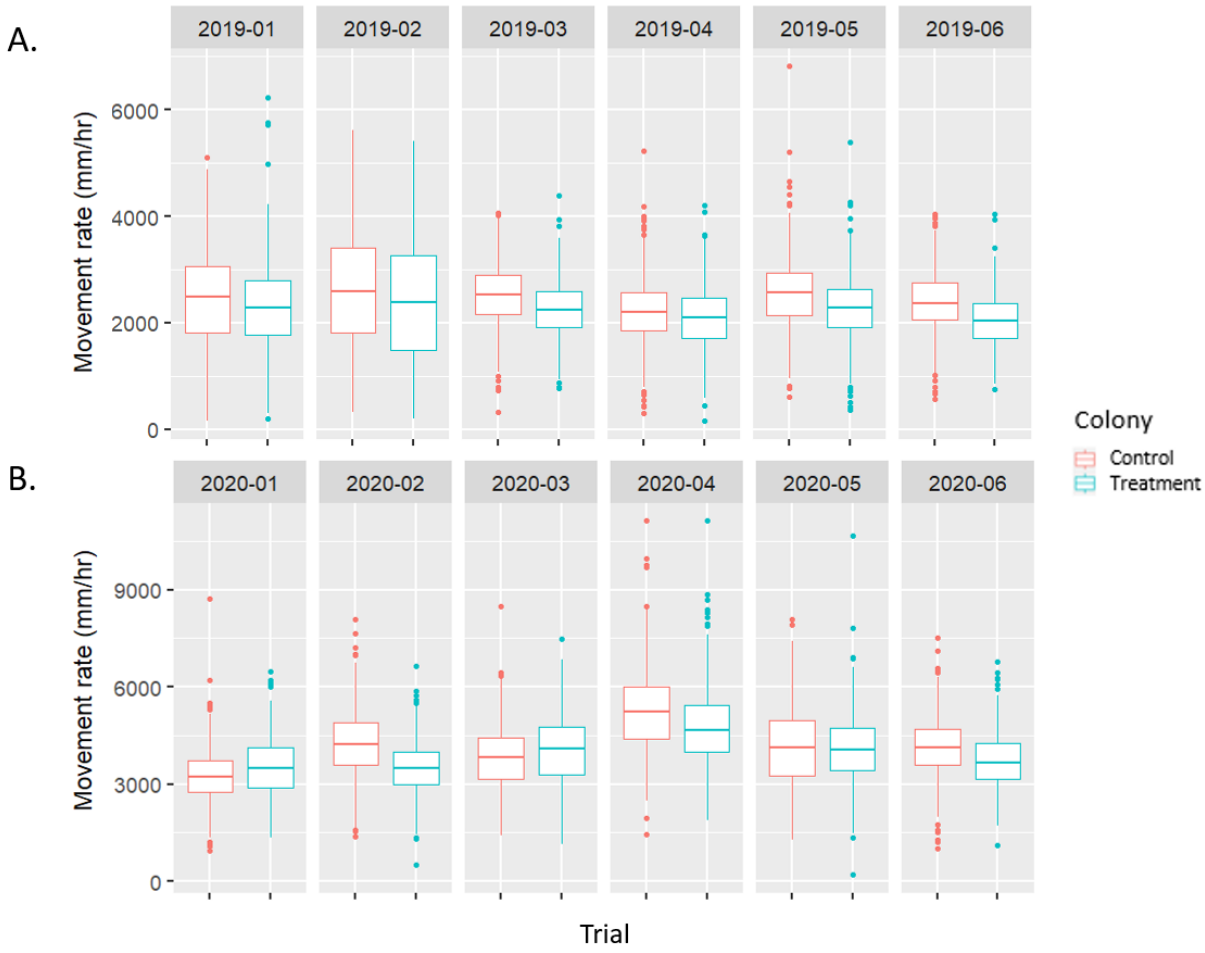

Figure 10 – Box plots of the normalized movement rates for bees in control and treatment colonies over the entire treatment period (box plots as in Fig. 9) for (A) Experiment I and (B) Experiment II, faceted by trial.

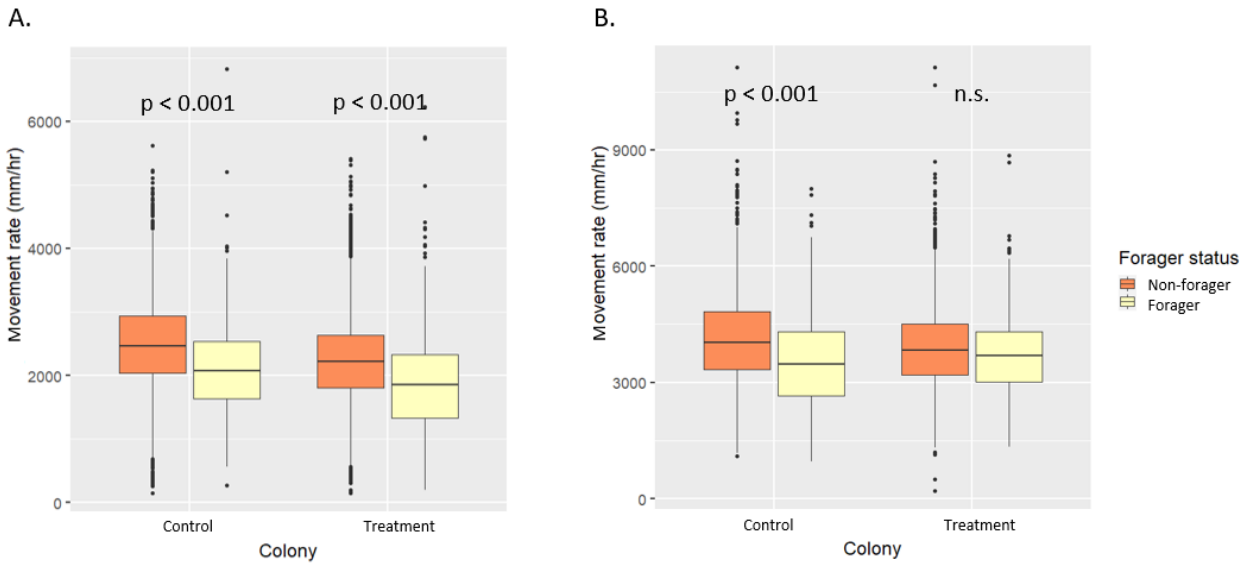

Figure 11 – Box plots of normalized movement rates for bees in control and treatment colonies, separated by task group (non-forager or forager) over the entire treatment period (box plots as in Fig. 9) for (A) Experiment I ($P < 0.001$ for control; $\exp(\beta) < 0.001$; n = 3804 for non-foragers, n = 480 for foragers; $P < 0.001$ for treatment; $\exp(\beta) < 0.001$; n = 3995 for non-foragers, n = 441 for foragers) and (B) Experiment II ($P < 0.001$ for control; $\exp(\beta) < 0.001$; n = 2796 for non-foragers, n = 290 for foragers; $P = 0.112$ for treatment; $\exp(\beta) < 0.001$; n = 2944 for non-foragers, n = 226 for foragers). n.s.: no statistically significant effect of treatment with $p > 0.05$.

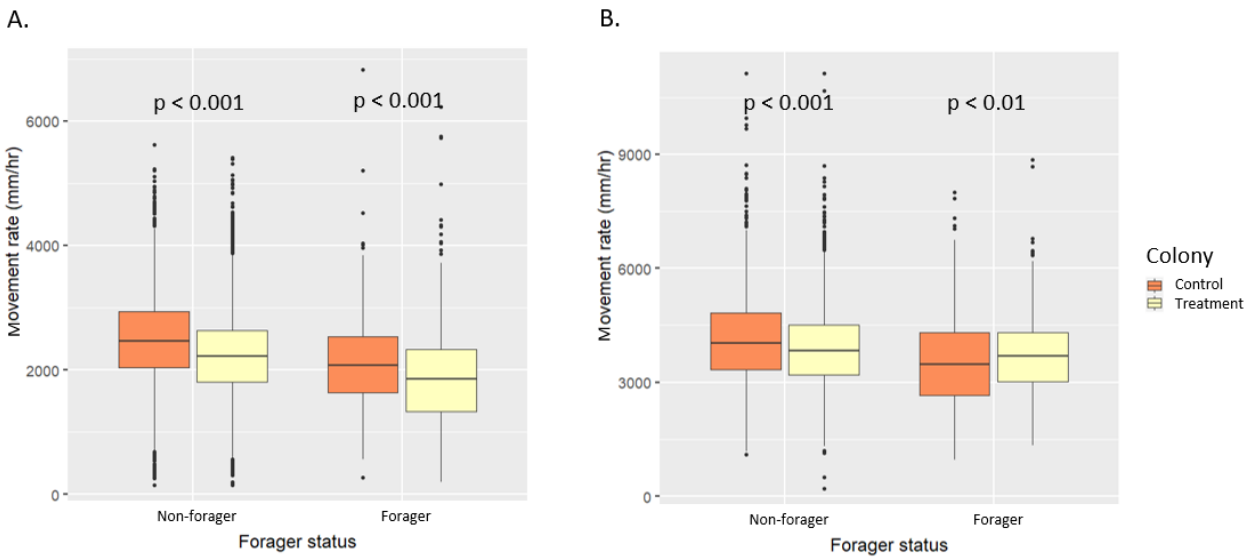

Figure 12 – Box plots of normalized individual movement rates for non-foragers and foragers in control or treatment colonies over the entire treatment period (box plots as in Fig. 9) for (A) Experiment I ($P < 0.001$ for non-foragers; $\exp(\beta) < 0.001$; $n = 3804$ for control, $n = 3995$ for treatment; $P < 0.001$ for foragers; $\exp(\beta) < 0.001$; $n = 480$ for control, $n = 441$ for treatment) and (B) Experiment II ($P < 0.001$ for non-foragers; $\exp(\beta) < 0.001$; $n = 2796$ for control, $n = 2944$ for treatment; $P < 0.01$ for foragers; $\exp(\beta) < 0.001$; $n = 290$ for control, $n = 226$ for treatment).

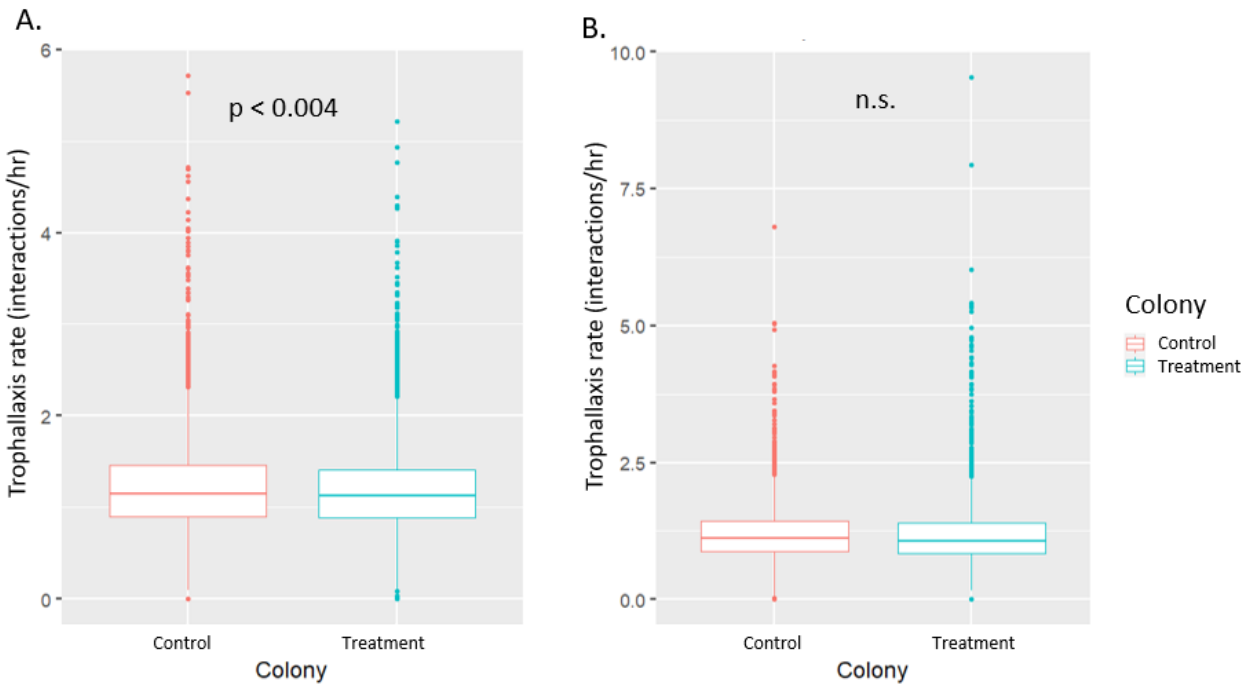

Figure 13 – Box plots of the normalized individual trophallaxis rates for bees in control and treatment colonies over the entire treatment period (box plots as in Fig. 9) for (A) Experiment I ($P < 0.004$; $\exp(\beta) = -0.006$; n = 4284 for control, n = 4436 for treatment) and (B) Experiment II ($P = 0.225$; $\exp(\beta) = -0.003$; n = 3086 for control, n = 3170 for treatment). n.s.: no statistically significant effect of treatment with $p > 0.05$.

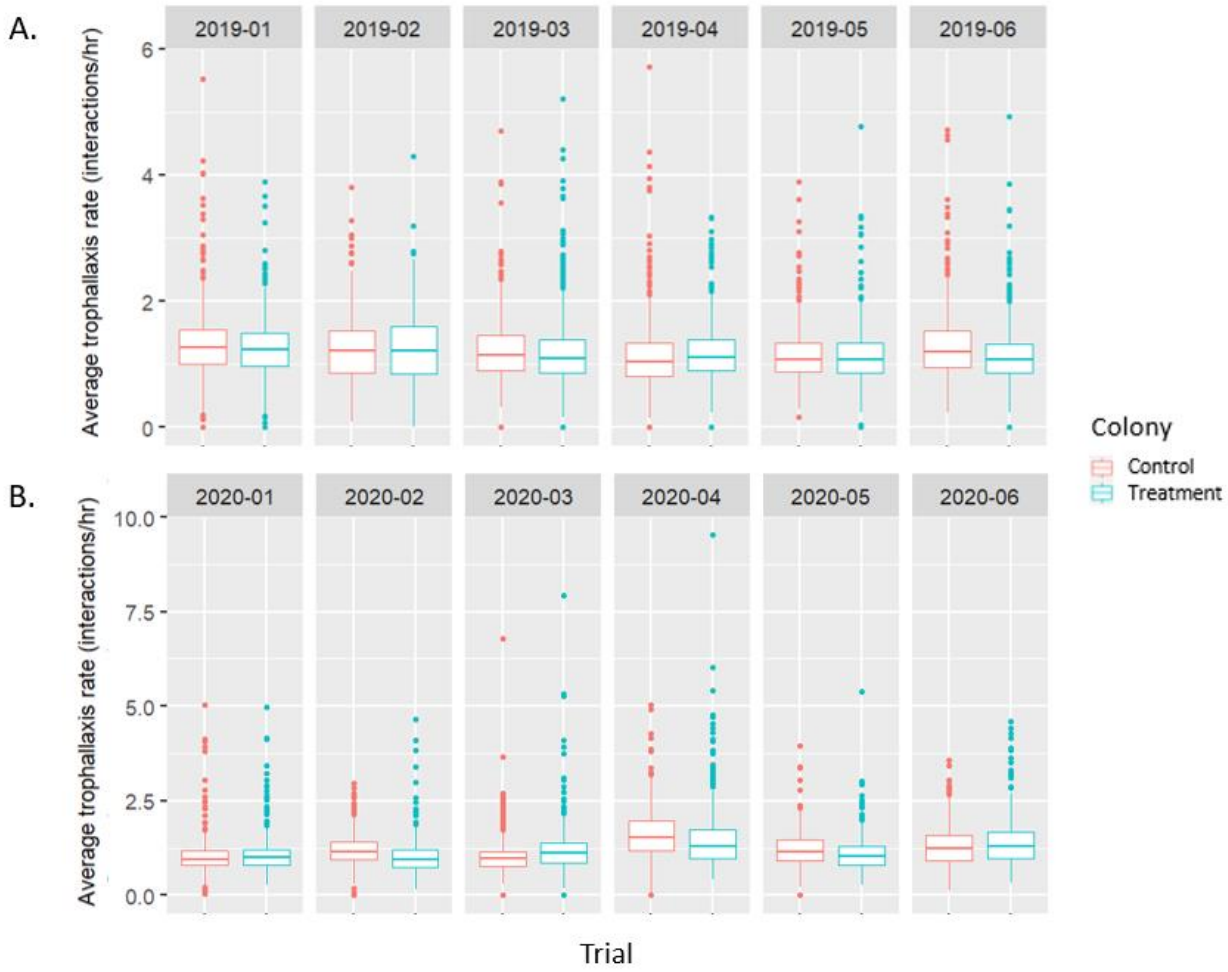

Figure 14 – Box plots of the normalized trophallaxis rates for bees in control and treatment colonies over the entire treatment period (box plots as in Fig. 9) for (A) Experiment I and (B) Experiment II, faceted by trial.

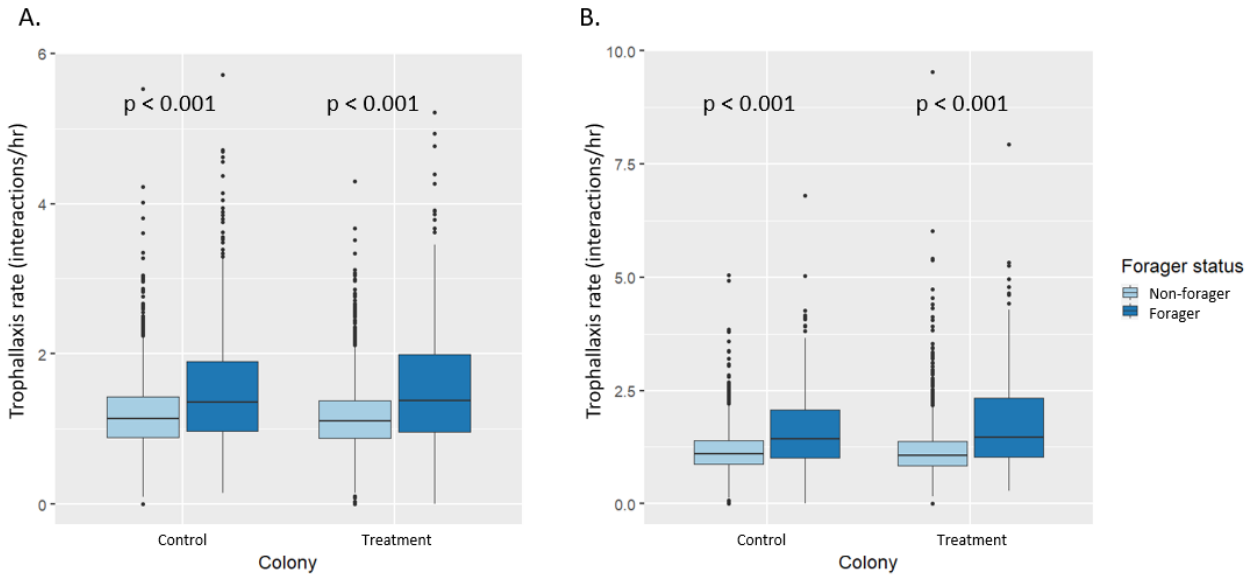

Figure 15 – Box plots of normalized trophallaxis rates for bees in control and treatment colonies, separated by task group (non-forager or forager) over the entire treatment period (box plots as in Fig. 9) for (A) Experiment I ($P < 0.001$ for control; $\exp(\beta) = 0.061$; n = 3804 for non-foragers, n = 480 for foragers; $P < 0.001$ for treatment; $\exp(\beta) = 0.068$; n =3995 for non-foragers, n = 441 for foragers) and (B) Experiment II ($P < 0.001$ for control; $\exp(\beta) = 0.069$; n = 2796 for non-foragers, n = 290 for foragers; $P < 0.001$ for treatment; $\exp(\beta) = 0.105$; n = 2944 for non-foragers, n = 226 for foragers).

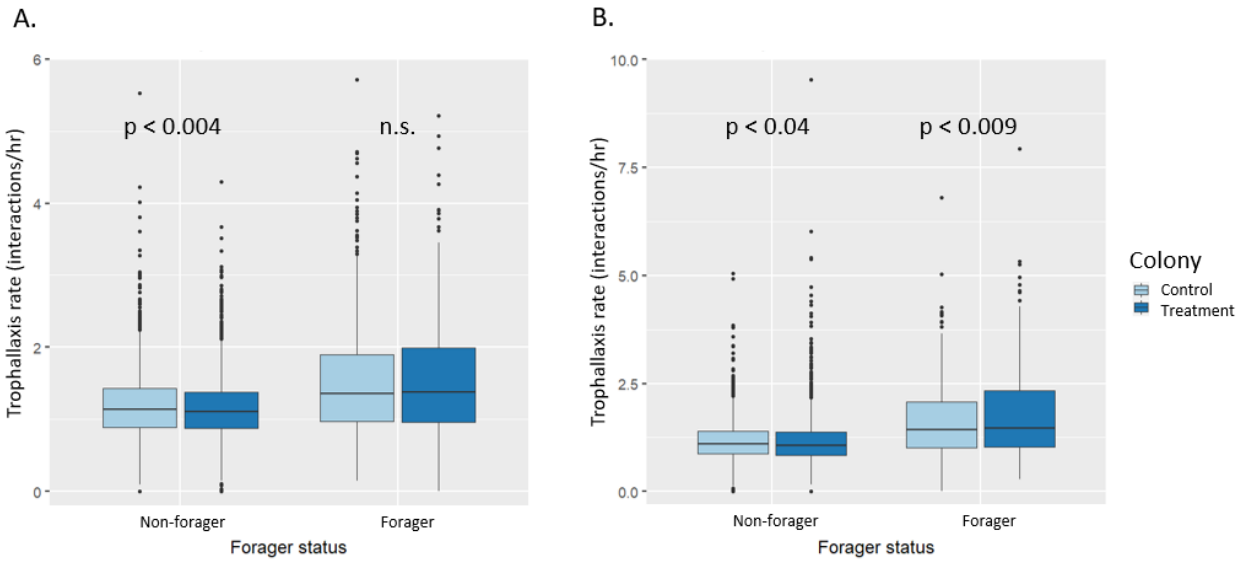

Figure 16 – Box plots of normalized trophallaxis rates for non-foragers or foragers, separated by colony (control or treatment) over the entire treatment period (box plots as in Fig. 9) for (A) Experiment I ($P < 0.004$ for non-foragers; $\exp(\beta) = -0.006$; $n = 3804$ for control, $n = 3995$ for treatment; $P = 0.443$ for foragers; $\exp(\beta) = 0.006$; $n = 480$ for control, $n = 441$ for treatment) and (B) Experiment II ($P < 0.04$ for non-foragers; $\exp(\beta) = -0.005$; $n = 2796$ for control, $n = 2944$ for treatment; $P < 0.009$ for foragers; $\exp(\beta) = 0.032$; $n = 290$ for control, $n = 226$ for treatment). n.s.: no statistically significant effect of treatment with $p > 0.05$.

Table 2 - Comparison of the trophallaxis network connectivity between treatment and control colonies over each treatment day. n.s.: no statistically significant effect of treatment with $p > 0.05$ ($n = 6$ for control, $n = 6$ for treatment).

Measurement	Treatment							
	Imidacloprid				Octopamine			
	Day 1	Day 2	Day 3	Day 4	Day 1	Day 2	Day 3	Day 4
Number of connected components	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Density	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean node strength	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Mean node degree	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Assortativity coefficient	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Clustering coefficient	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.
Degree distribution entropy	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.	n.s.

Table 3 – Summary of individual-level results from this study. Direction reported is in response to treatment. n.s.: no statistically significant effect of treatment with $p > 0.05$

	EXPERIMENT I (IMIDACLOPRID)		***EXPERIMENT II (OCTOPAMINE)***	
	Movement rate	***Trophallaxis rate***	***Movement rate***	***Trophallaxis rate***
NON-FORAGERS	Decrease	Decrease	Decrease	Decrease
FORAGERS	Decrease	n.s.	Increase	Increase
WHOLE-COLONY	Decrease	Decrease	Decrease	n.s.

REFERENCES

Aliouane, Y., el Hassani, A.K., Gary, V., Armengaud, C., Lambin, M., & Gauthier, M. (2009). Subchronic exposure of honeybees to sublethal doses of pesticides: effects on behavior. *Environ Toxicol Chem, 28*, 113–122.

Barron, A.B., & Robinson, G.E. (2005). Selective modulation of task performance by octopamine in honey bee (*Apis mellifera*) division of labor. *J Comp Physiol A, 191*, 659-668.

Barron, A.B., Maleszka, R., vander Meer, R.K., & Robinson, G.E. (2007). Octopamine modulates honey bee dance behavior. *PNAS, 104*, 1703-1707.

Baum, L.E. (1972). A maximization technique occurring in the statistical analysis of probabilistic functions of Markov chains. *Ann Math Stat, 41*, 164-171.

Bibi, S., Husain, S.Z., & Malik, R.N. (2008). Pollen analysis and heavy metals detection in honey samples from selected countries. *Pak J Bot, 40*, 507-516.

Bogdanov, S. (2006). Contaminants of bee products. *Apidologie, 37*, 1-18.

Boulay, R., Soroker, V., Godzinska, E.J., Hefetz, A., & Lenoir, A. (2000). Octopamine reverses the isolation-induced increase in trophallaxis in the carpenter ant *Camponotus fellah*. *J Exp Biol, 203*, 513-520.

Bromenshenk, J.J., Henderson, C.B., Seccomb, R.A., Welch, P.M., Debnam, S.E., & Firth, D.R. (2015). Bees as biosensors: Chemosensory ability, honey bee monitoring systems, and emergent sensor technologies derived from the pollinator syndrome. *Biosensors, 5*, 678-711.

Costa, L.F., Rodriques, F.A., Travieso, G., & Billas Boas, P.R. (2007). Characterization of complex networks: A survey of measurements. *Adv in Phys, 56*, 167-242.

Crailsheim, K. (1998). Trophallactic interactions in the adult honeybee (*Apis mellifera L.*). *Apidologie, 29*, 97-112.

Crall, J.D., Gravish, N., Mountcastle, A.M., & Combes, S.A. (2015). BEEtag: A low-cost, image-based tracking system for the study of animal behavior and locomotion. *PLoS One, 10*: e0136487.

Cremer, S., Pull, C.D., & Fürst, M.A. (2018). Social immunity: Emergence and evolution of colony-level disease protection. *Annu Rev of Entomol, 63*, 105-123.

Cresswell, J.E. (2011). A meta-analysis of experiments testing the effects of a neonicotinoid insecticide (imidacloprid) on honey bees. *Ecotoxicology, 20*, 149–157.

Decourtye, A., Lacassie, E., & Pham-Delègue, M.H. (2003). Learning performances of honeybees (*Apis mellifera* L.) are differentially affected by imidacloprid according to the season. *Pest Manage Sci, 59,* 269-278.

Decourtye, A., Devillers, J., Cluzeau, S., Charreton, M., & Pham-Delègue, M.H. (2004). Effects of imidacloprid and deltamethrin on associative learning in honeybees under semi-field and laboratory conditions. *Ecotoxicol and Environ Saf, 57,* 410-419.

Decourtye, A., Devillers, J., Aupinel, P., Brun, F., Bagnis, C., Fourrier, J., & Gauthier, M. (2011). Honeybee tracking with microchips: a new methodology to measure the effects of pesticides. *Ecotoxicology, 20,* 429-437.

De Miranda, J.R. & Genersch, E. (2010). Deformed wing virus. *J Invertebr Pathol, 103 (Suppl 1),* S48-S61.

Dively, G. P., Embrey, M. S., Kamel, A., Hawthorne, D. J., & Pettis, J. S. (2015). Assessment of chronic sublethal effects of imidacloprid on honey bee colony health. *PLoS One, 10,* e0118748.

Dolezal, A.G., Corrillo-Tripp, J., Miller, W.A., Bonning, B.C., & Toth, A.L. (2015). Pollen contaminated with field-relevant levels of cyhalothrin affects honey bee survival, nutritional physiology, and pollen consumption behavior. *J Econ Entomol.* 109(1), 41-48.

Grüter, C., & Farina, W.M. (2009). Trophallaxis: A mechanism of information transfer. *Food Exploitation by Social Insects*. eds Jarau, S., Hrncir, M. CRC. 183-197pp.

Guzmán-Novoa, E., Page, R.E., & Gary, N.E. (1994). Behavioral and life-history components of division of labor in honey bees (*Apis mellifera* L.). *Behav Ecol Sociobiol, 34,* 409-417.

ECCC and Health Canada. (2017). Screening assessment: Selenium and its compounds.

Evans, J.D., Aronstein, K., Chen, Y.P., Hetru, C., Imler, J-L, Jiang, H., Kanost, M., Thompson, G.J., Zou, Z., & Hultmark, D. (2006). Immune pathways and defense mechanisms in honey bees (*Apis mellifera*). *Insect Mol Biol.* 15(5), 645-56.

Faucon, J.P., Aurières, C., Drajnudel, P., Mathieu, L., Ribière, M., Martel, A.C., Zeggane, S., Chauzat, M.P., & Aubert, M.F. (2005). Experimental study on the toxicity of imidacloprid given in syrup to honey bee (*Apis mellifera*) colonies. *Pest Manage Sci, 61,* 111-125.

Fine, J.D., Torres, K.M., Martin, J., & Robinson, G.E. (2021). Assessing agrochemical risk to mated honey bee queens. *J Vis Exp,* (169), e62316

Free, J.B. (1956). A study of the stimuli which release the food begging and offering responses of worker honeybees. *Br J Anim Behav, 4*, 94-101.

Free, J.B. (1964). The allocation of duties among worker honeybees. *Animal Behavior, 12*, 389-390.

Geffre, A., Gernat, T., Harwood, G., Jones, B., Gysi, D.M., Hamilton, A.R., Bonning, B.C., Toth, A., Robinson, G.E., & Dolezal, A.G. (2020). Honey bee virus causes context-dependent changes in host social behavior. *PNAS, 117*, 10406-10413.

Gernat, T., Rao, V.D., Middendorf, M., Dankowicz, H., Goldenfeld, N., & G.E. Robinson. (2018). Automated monitoring of behavior reveals bursty interaction patterns and rapid spreading dynamics in honeybee social networks. *PNAS, 115*, 1433–1438.

Gernat, T., Jagla, T., Jones, B.M., Middendorf, M., & Robinson, G.E. (2020). Automated monitoring of animal behaviour with barcodes and convolutional neural networks. *bioRxiv*.

Ghosh, S., Jeon, H., & Jung, C. (2020). Foraging behaviour and preference of pollen sources by honey bee (*Apis mellifera*) relative to protein contents. *J Ecol and Environ, 44*, 4.

Gilley, D.C. (1998). The identity of nest-site scouts in honey bee swarms. *Apidologie, 29*, 229-240.

Greenwald, E., Segre, E., & Feinerman, O. (2015). Ant trophallactic networks: Simultaneous measurement of interaction patterns and food dissemination. *Sci Rep, 5*, 12496.

Greenwald, E., Eckmann, J., & Feinerman, O. (2019). Colony entropy – Allocation of goods in ant colonies. *PLoS Comput Bio, 15*: e1006925.

Gregorc, A., Alburaki, M., Rinderer, N., Sampson, B., Knight, P.R., Shahid, K., & Adamczyk, J. (2018). Effects of coumaphos and imidacloprid on honey bee (Hymenoptera: Apidae) lifespan and antioxidant gene regulations in laboratory experiments. *Sci Rep, 8*, 15003.

Hladun, K.R., Smith, B.H., Mustard, J.A., Morton, R.R., & Trumble, J.T. (2012). Selenium toxicity to honey bee (*Apis mellifera* L.) pollinators: effects on behaviors and survival. *PLoS One 7*(4).

Jones, B.M., Rao, V.D., Gernat, T., Jagla, T., Cash-Ahmed, A.C., Rubin, B.E.R., Comi, T.J., Bhogale, S., Husain, S.S., Blatti, C., Middendorf, M., Sinha, S., Chandrasekaran, S., & Robinson, G.E. (2020). Individual differences in honey bee behavior enabled by plasticity in brain gene regulatory networks. *eLife.*

Jones, K.C. (1987). Honey as an indicator of heavy metal contamination. *Water Air Soil Poll, 33*, 179-189.

Kessler, S.C., Tiedeken, E.J., Simcock, K.L., Derveau, S., Mitchell, J., Softley, S., Stout, J.C., & Wright, G.A. (2015). Bees prefer foods containing neonicotinoid pesticides. *Nature, 521,* 74–76.

Kirchner, W.F., & Landauer, M. (1994). The causes of the tremble dance of the honeybee, *Apis mellifera. Behav Ecol Sociobiol, 35*, 303-308.

Kirchner, W.H. (1999). Mad-bee-disease? Sublethal effects of imidacloprid ("Gaucho") on the behavior of honey-bees. *Apidologie. 30*, 422.

Krupke, C.H., Hunt, G.J., Eitzer, B.D., Andino, G., & Given, K. (2012) Multiple routes of pesticide exposure for honey bees living near agricultural fields. *PLoS One, 7*(1).

Lambin, M., Armengaud, C., Raymond, S., & Gauthier, M. (2001). Imidacloprid-induced facilitation of the proboscis extension reflex habituation in the honeybee. *Insect Biochem Physiol, 48,* 129-134.

Lecocq, A., Jensen, A.B., Kryger, P., & Nieh, J.C. (2016) Parasite infection accelerates age polyethism in young honey bees. *Sci Rep, 6,* 22042.

LeBoeuf, A., Waridel, P., Brent, C., Goncalves, A., Menin, L., Ortiz, D., Riba-Grognuz, O., Koto, A., Soares, Z., Privman, E., Miska, E., Benton, R., & Keller, L. (2016). Oral transfer of chemical cues, growth proteins and hormones in social insects. *eLife.*

Leita, L., Muhlbachova, G., Cesco, S., Barbattini, R., & Mondini, C. (1996). Investigation of the use of honey bees and honey bee products to assess heavy metals contamination. *Environ Monit and Assess, 43,* 1-9.

Liang, Z.S., Nguyen, T., Mattila, H.R., Rodriguez-Zas, S.L., Seeley, T.D., & Robinson, G.E. (2012). Molecular determinants of scouting behavior in honey bees. *Science, 335*, 1225-1228.

Lindauer, M. (1952). Ein Beitrag zur Frage der Arbeitsteilung im Bienenstaat. *Z Vgl Physiol* 34, 299-345.

Lindauer, M. (1954). Temperaturregulierung und Wasserhaushalt im Bienenstaat. *Z Vgl Physio* 36, 391-432.

Linn, M., Glaser, S.M., Peng, T., & Grüter, C. (2020). Octopamine and dopamine mediate waggle dance following and information use in honeybees. *Proc Biol Sci, 287*.

Medrzycki, P., Montanari, R., Bortolotti, L., Sabatini, A.G., Maini, S.T., & Porrini, C. (2003). Effects of imidacloprid administered in sub-lethal doses on honey bee behavior. Laboratory tests. *Bull Insectol, 56,* 59-62.

Menzel, R., Steinmann, E., Souza, J.D., & Backhaus, W. (1988). Spectral sensitivity of photoreceptors and colour vision in the solitary bee, *Osmia rufa*. *J Exp Biol, 136*, 35-52.

Mersch, D.P., Crespi, A., & Keller, L. (2013). Tracking individuals shows spatial fidelity is a key regulator of ant social organization. *Science, 340*, 1090-1093.

Nixon, H.L., & Ribbands, C.R. (1952). Food transmission within the honeybee community. *Proc R Soc Lond B Biol Sci, 140*, 43-50.

Park , W. (1925). The storing and ripening of honey by honeybees. *J Econ Entomol, 18*, 405-410.

Peng, T., Schroeder, M., & Grüter, C. (2020). Octopamine increases individual and collective foraging in a neotropical stingless bee. *Biol Lett, 16*.

Pernal, S.F., & Currie, R.W. (2001). The influence of pollen quality on foraging behaviour in honeybees (*Apis mellifera* L.). *Behav Ecol Sociobiol*, 51, 53-68.

Roberts, S.P., & Elekonich, M.M. (2005). Muscle biochemistry and the ontogeny of flight capacity during behavioral development in the honey bee, *Apis mellifera. J Exp Biol, 208*, 4193-4198.

Ribière, M., Olivier, V., & Blanchard, P. (2010). Chronic bee paralysis: A disease and a virus like no other? *J Invertebr Pathol, 103 (Suppl 1)*, S120-S131.

Robinson, G.E., Page, R.E. Jr., Strambi, C., & Strambi, A. (1989). Hormonal and genetic control of behavioral integration in honey bee colonies. *Science, 246*, 109-112.

Robinson, G.E. (1992). Regulation of division of labor in insect societies. *Annu Rev Entomol, 37*, 637-665.

Sattelle, D.B., & Breert, H. (1990). Cholinergic nerve terminals in the central nervous system of insects. *J Neuroendocrinol, 2*.

Scheiner, R., Plückhahn, S., Öney, B., Blenau, W., & Erber, J. (2002). Behavioural pharmacology of octopamine, tyramine and dopamine in honey bees. *Behav Brain Res, 136*, 545-553.

Schulz, D.J., & Robinson, G.E. (1999). Biogenic amines and division of labor in honey bee colonies: behaviorally related changes in the antennal lobes and age-related changes in the mushroom bodies. *J Comp Physiol A, 184*, 481-488.

Schulz, D.J., & Robinson, G.E. (2001). Octopamine influences division of labor in honey bee colonies. *J Comp Physiol A, 187*, 53-61.

Schulz, D.J., Barron, A.B., & Robinson, G.E. (2002). A role for octopamine in honey bee division of labor. *Brain Behav Evol, 60,* 350-435.

Schulz, D.J., Sullivan, J.P., & Robinson, G.E. (2002). Juvenile hormone and octopamine in the regulation of division of labor in honey bee colonies. *Horm Behav, 42,* 222-231.

Schulz, D.J., Elekonich, M.M., & Robinson, G.E. (2003). Biogenic amines in the antennal lobes and the initiation and maintenance of foraging behavior in honey bees. *J Neurobiol, 54,* 406–416.

Seeley, T.D. (1986). Social foraging in honey bees how colonies allocate foragers among patches of flowers. *Behav Ecol Sociobiol, 19,* 343-354.

Seeley, T.D. (1995). *Wisdom of the Hive: The Social Physiology of Honey Bee Colonies.* Harvard University Press. 318pp.(4).

Sivakoff, F.S., Prajzner, S.P., & Gardiner, M.M. (2020). Urban heavy metal contamination limits bumblebee colony growth. *J Appl Ecol 57*(8), 1561-1569.

Stroeymeyt, N., Casillas-Pérez, B., & Cremer, S. (2014). Organizational immunity in social insects. *Curr Opin Insect Sci, 5,* 1-15.

Stroeymeyt, N., Grasse, A.V., Crespi, A., Mersch, D.P., Cremer, S., & Keller, L. (2018). Social network plasticity decreases disease transmission in a eusocial insect. *Science, 362,* 941-945.

Tenczar, P., Lutz, C.C., Rao, V.D., Goldenfeld, N., & Robinson, G.E. (2014). Automated monitoring reveals extreme interindividual variation and plasticity in honeybee foraging activity levels. *Animal Behavior, 95,* 41-48.

Tomizawa, M., & Casida, J.E. (2005). Neonicotinoid insecticide toxicology: Mechanisms of Selective Action. *Annu Rev Pharmacol Toxicol* 45:1, 247-268.

Traniello, I., Hamilton, A., Gernat, T., Cash-Ahmed, A.C., Harwood, G.P., Ray, A.M., Glavin, A., Torres, J., Goldenfeld, N., & Robinson, G.E. (2022). Context-dependent influence of threat on honey bee social network dynamics and brain gene expression. *J Exp Biol, 225*.

Visscher, P., & Seeley, T. (1982). Foraging strategy of honeybee colonies in a temperate deciduous forest. *Ecology, 63,* 1790-1801.

Viterbi, A.J. (1967). Error bounds for convolutional codes and an asymptotically optimum decoding algorithm. *IEEE Transact Inf Theory, 3,* 260-269.

Wagener-Hulme, C., Kuehn, J.C., Schulz, D.J., & Robinson, G.E. (1999) Biogenic amines and division of labor in honey bee colonies. *J Comp Physiol A, 184,* 471-479.

Wario, F., Wild, B., Couvillon, M.J., Rojas, R., & Landgraf, T. (2015). Automatic methods for long-term tracking and the detection and decoding of communication dances in honeybees. *Front Ecol Evol, 3*, 103.

Williamson, S.M., Willis, S.J., & Wright, G.A. (2014). Exposure to neonicotinoids influences the motor function of adult worker honeybees. *Ecotoxicology, 23*, 1409-1418.

Winston, M.L. (1991). *The Biology of the Honey Bee.* Harvard University Press.

Wright, G.A., Baker, D.D., Palmer, M.J., Stabler, D., Mustard, J.A., Power, E.F., Borland, A.M., & Stevenson, P.C. (2013). Caffeine in floral nectar enhances a pollinator's memory of reward. *Science.* 339(6124), 1202-1204.

Wu-Smart, J., & Spivak, M. (2016). Sub-lethal effects of dietary neonicotinoid insecticide exposure on honey bee queen fecundity and colony development. *Sci Rep 6*: 32108.