Practical Project 2018/2019
(School of Biological Sciences)

Title of Project: Exposure effects of neonicotinoid imidacloprid on foraging behavior, pollination behavior and mortality rates of *Bombus terrestris*

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Abstract

Imidacloprid, belonging to the neonicotinoid insecticides, is widely used in agriculture to deter predation on crops. Neonicotinoids are a systemic pesticide and have a propensity to translocate to flowers and other non-target agricultural crops foraged by pollinators making them a non-target risk. This study subjected *Bombus terrestris* to three levels of dietary exposure: control, field-realistic dose and a sub-lethal high-dose. Throughout the four-week study observations of foraging behaviors, active population densities and cumulative weekly death rates were collected. This study aims to highlight the necessity to promote better management of wild pollinators by outlining the risks faced through the growing use of pesticides such as imidacloprid. Also to better inform the argument if the cost of these pesticides outweighs the benefits. Our research found significant differences in mortality rates, overall flower visitations and active population densities directly linked to increased exposure to imidacloprid.

Keywords: bumble bees, imidacloprid, neonicotinoid, colony A, colony B, colony C.

Introduction

Pollinator decline is presently widely studied and has been directly linked to factors such as habitat loss, invasive species and, to the interest of this study, pesticides (Gill and Raine, 2014) (Potts *et al*., 2010). Many pollinators, including bumblebees, have become nontarget risks (Wu-Smart and Spivak, 2017) throughout increased use of systemic translocating pesticides. The effects of this increased usage is important to monitor as bumble bees are integral to maintaining ecological balances of wildflower ecosystems and also many ecosystems associated with agricultural production (Ricketts *et al*., 2008).

Bombus terrestris, and other wild pollinators, are not considered the flagship species of insect pollinators; this title belongs to the honeybee and has been this way for many decades. This has lead to an over-reliance on the species and a poor understanding and management of wild

pollinators and their role in agricultural pollination as well as the risks to them with regards to neonicotinoids. With this in mind, if the habitats of the bumblebee are managed correctly they could play a vital role in filling the pollination deficits created by the overpopularisation of honeybees (Julier and Roulston, 2009). Honeybees have been purchased for, but are ineffective at, pollinating deep flowers and buzz-pollinated crops (Goulson, 2003). Published research (Willmer, Bataw and Hughes, 1994) has also shown that non-native honeybees, unlike endemic bumblebees, will not continue to forage and pollinate under poor weather conditions, such as the cold and rain. This is important when considering many agricultural practices, such as apple orchard farming, that rely on honeybee pollination in areas that regularly experience poor weather conditions during flowering times (Goulson, 2003).

We hypothesise that the imidacloprid pesticide will have significant detrimental impacts on the colonies' foraging behavior and activity. We also predict there will be a higher rate of mortality in colonies undergoing exposure treatments: highest in colony C, our colony treated with a high-level imidacloprid dosage.

Methods

Three colonies were purchased from Agralan and were assigned their treatments at random: 0 µg per litre or 0 ppb (named colony **A**), 6 µg per litre or ppb (named colony **B**) and 12 µg per litre or ppb (named colony **C**), in October 2018. These concentrations were an estimation of likely nectar concentrations based on previous research (Laycock *et al.*, 2012) (Dively & Kamel, 2012), doubling this to get the high dose. Three colonies were connected to individual 40x40x60 cm flight arenas via a small tube. Colonies were maintained in the same room to ensure identical temperature, 23°C, and light exposure, a 12 hour light cycle. All three colonies received 2 tsp of pollen to supplement their dietary requirements directly into the colony boxes on Mondays, Wednesdays and Fridays.

For the non-control treatments, 6 µg imidacloprid was added to 600 ml of sugar solution to make it 6 ppm and 12 µg was added to create the high-dose. The treatments were supplied via the medium of custom made flowers every two days. Blue and yellow laminated card discs, with a small circle cut out in the middle to feed a cotton gauze wick through, were fixed on to the top of a specimen tube. Two blue and two yellow were placed in to each flight arena, arranged randomly using a 12-sided dice on identical 3x4 grids.

Visitation counts were made on Mondays, Wednesdays and Fridays and foraging durations were timed every friday for two hours per observation per colony. Both data sets were to be analysed by a multi-way ANOVA taking into account the day of the experiment, the flower colour and the colony. Deaths were recorded and collected from the flight arenas at the beginning of each of these observational period and active population densities were recorded daily. Both of these were to be analysed by two-way ANOVA taking into account the day of the experiment and the imidacloprid dosage. All deceased bees during the experiment and living bees at the

termination of the experiment were frozen and were measured to the nearest mm. The means, standard deviations and variances were calculated by colony.

Results

Time spent foraging

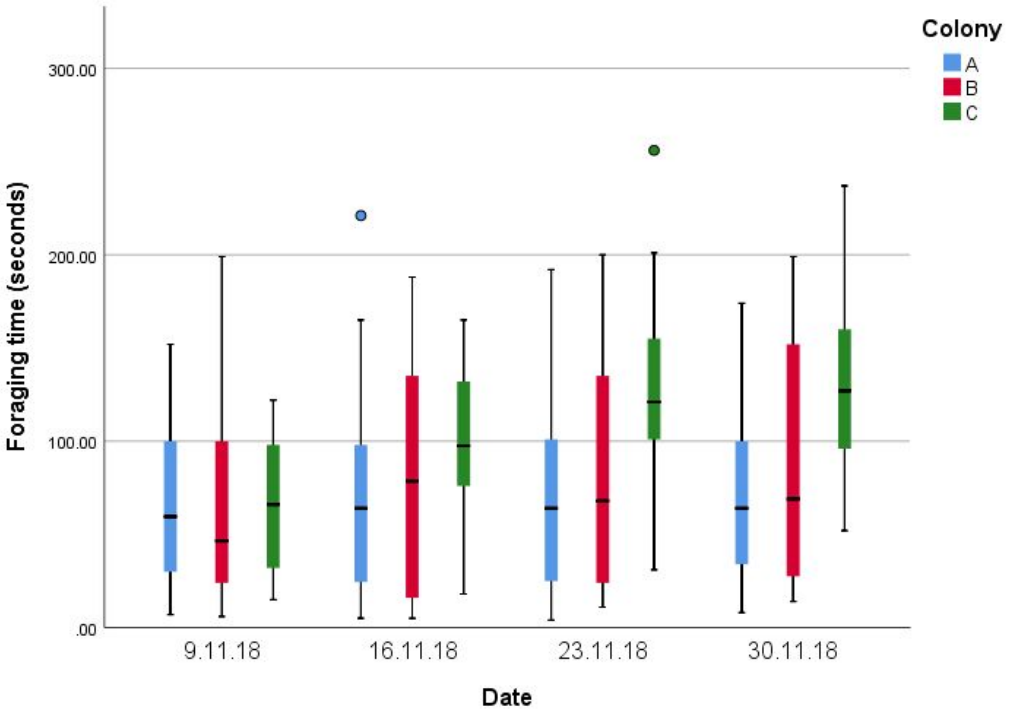

Figure 1: The time taken by bees to forage on the flowers in the flight arenas of the three colonies. Colonies A, B, and C are the control colony (0 µg L^{-1} imidacloprid), field-dose colony (6 µg L^{-1} imidacloprid) and high-dose colony (12 µg L^{-1} imidacloprid) respectively.

The data were found to be parametric and so were analysed by multi-way ANOVA. Significant differences were found in the foraging time due to both the duration of the experiment, $F=1809960$, $d.f.=3$, $p<0.001$, as well as the imidacloprid dosage, $F=30.9458$, $d.f.=2$, $p<0.001$. A Bonferroni post hoc test determined that there were significant differences in the foraging time between all colonies. Between colonies A and B $p=0.005$; between colonies A and C $p<0.001$, and between colonies B and C $p<0.001$. Flower colour was not found to have made a significant difference to the time spent foraging as $F=0.0256$, $d.f.=1$, $p=0.873$.

Visitations over time

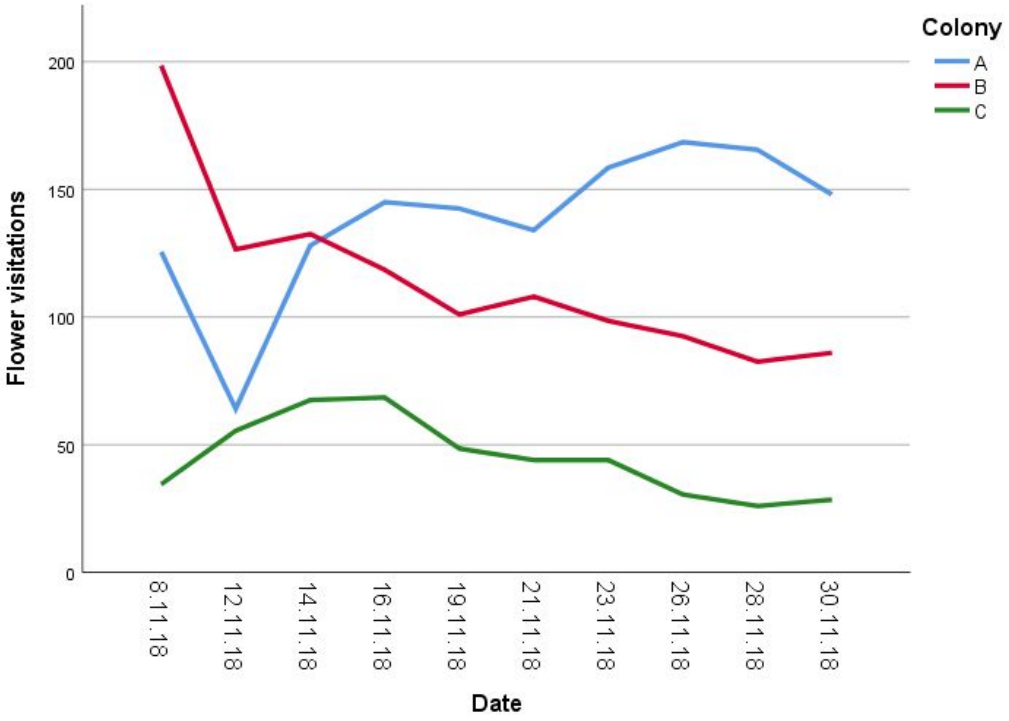

Figure 2: *Bombus terrestris* flower visitations over a two hour period as measured twice a week, separated by colony. Colonies A, B, and C are the control colony (0 µg L^{-1} imidacloprid), field-dose colony (6 µg L^{-1} imidacloprid) and high-dose colony (12 µg L^{-1} imidacloprid) respectively.

The data were found to be parametric and so were analysed by multi-way ANOVA. There was found to be a significant difference in the number of flower visitations over time as F=23.716, d.f.=1, p=0.132, as well as a significant difference in the number of flower visitations due to the imidacloprid dosage as F=2.828, d.f.=9, p=0.028. This analysis was followed up with a Bonferroni post hoc test that found a significant difference in flower visitations between all of the colonies. Between colonies A and B p=0.019; between colonies A and C p<0.001, and between colonies B and C p<0.001.

Active population densities

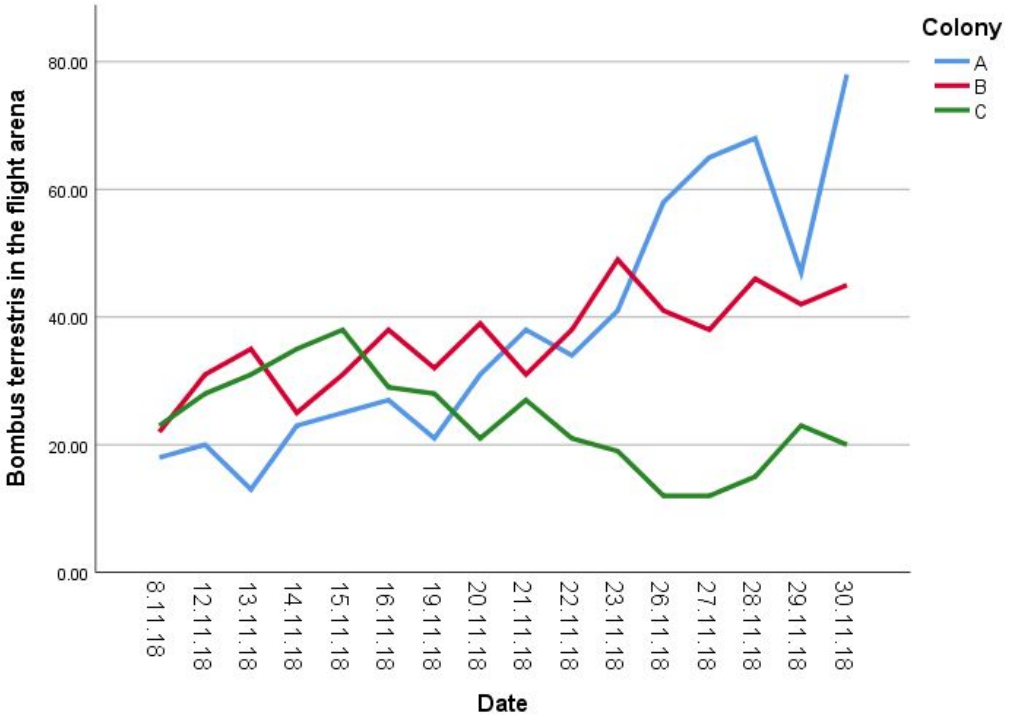

Figure 3: An approximate count of the number of *Bombus terrestris* in the flight arenas of the three colonies. Colonies A, B, and C are the control colony (0 μg L^{-1} imidacloprid), field-dose colony (6 μg L^{-1} imidacloprid) and high-dose colony (12 μg L^{-1} imidacloprid) respectively.

The data were found to be parametric and so were analysed by two-way ANOVA. A significant result was found for the effect of the imidacloprid dosage on the number of bees in the flight arenas, $F=3.571$, $d.f.=2$, $p=0.041$. Subsequently a Bonferroni post hoc test was carried out which determined that, while there was no significant difference in the number of bees in the flight arena between the control dose colony and the field dose colony, $p=0.746$, there were significant differences between the control dose and high dose, $p=0.011$, and between the high dose and field dose, $p=0.018$.

Deaths

During the course of the experiment dead bees were collected from the flight arena. At the close of the experiment the total number of bees were counted in each colony and the proportion of dead bees counted for each. The proportions of the colonies that died were counted to be 0.050 for the control dose colony, 0.134 for the field dose colony and 0.223 for the high dose colony.

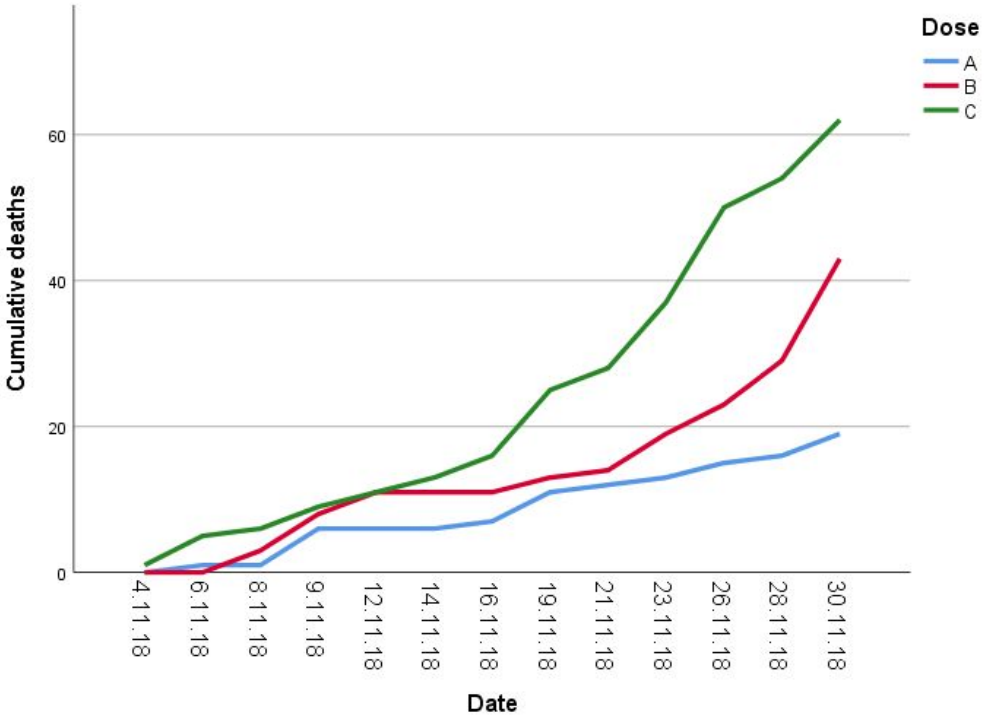

Figure 4: *Bombus terrestris* deaths shown cumulatively by colony. Colonies A, B, and C are the control colony (0 µg L^{-1} imidacloprid), field-dose colony (6 µg L^{-1} imidacloprid) and high-dose colony (12 µg L^{-1} imidacloprid) respectively.

The data were found to be parametric and so were analysed by two-way ANOVA. Time was shown to have a significant effect on *Bombus terrestris* deaths as $F=8.323$, $d.f.=12$, $p<0.001$. A significant result was also found for the effect of imidacloprid dosage on death numbers. The subsequent Bonferroni post hoc test found significant differences between all of the colonies. Between colonies A and B $p=0.011$; between colonies A and C $p=0.023$, and between colonies B and C $p=0.018$.

Population count at termination.

Table 1: Total Bombus terrestris population count and lengths. Colonies A, B, and C are the control colony (0 μg L^{-1} imidacloprid), field-dose colony (6 μg L^{-1} imidacloprid) and high-dose colony (12 μg L^{-1} imidacloprid) respectively.

Colony	Total	Range	Minimum Length (mm)	Maximum Length (mm)	Mean	Standard Deviation	Variance
A	358	21	5	26	15.149	4.407	19.423
B	277	18	7	25	14.033	3.399	11.553
C	247	17	9	26	14.827	4.091	16.735

The data were found to be parametric and so were analysed by one-way ANOVA. The imidacloprid dosage was found to have a significant effect on the population size as $F=4.479$, $d.f.=20$, $p<0.001$. The subsequent Bonferroni post hoc test found significant differences between colonies A and B, $p=0.001$ and between colonies A and C, $p<0.001$, but found no significant difference between colonies B and C.

Discussion

Time spent foraging

There were significant differences in the time spent foraging between all colonies (Figure 1). All colonies displayed short feeding times per plant at the start of the experiment which we predicted was a result of explorative behavior to a new environment; described as short or long orientational flights (Degen *et al*., 2015). Over the course of the experiment both treatment colonies were found to spend significantly more time foraging on each flower however we believe they were likely apathetic towards foraging and were generally being docile near where they might feed, a theory shared in other research (Mommaerts *et al*., 2010). Colony A showed no signs of apathetic behavior towards foraging and maintained a consistent time foraging per flower throughout.

Visitations

We found significant evidence that both treatment colonies visited less flowers, when compared to one another and to colony A, over the course of the exposure to imidacloprid (Figure 2). This fits with similar research (Fauser-Misslin *et al.*, 2013) which has shown that bumblebee performance will decrease when exposed to imidacloprid. Colony C shows behavior described previously (Suchail, Guez and Belzunces, 2001) from the onset of acute exposure to the

pesticide wherein the colony exhibits an immediate state of hyperactivity followed by a steady decline to hypoactivity of the colony. Additionally, this fits with the mathematical modelling of pesticide-induced sublethal stress to bee colonies (Bryden *et al*., 2013).

Active population densities

It is important to be aware colonies will be in different levels of maturity when delivered to us for the experiment and this can potentially impact the active populations in the flight arenas, such as stages of maturity or hibernation. Notwithstanding, our data (Figure 3) showed a significant incline in the active population density of bees in colony A within the flight arena. We predict the increase in this colony's activity to be due to familiarisation with their environment and reproduction and maturation of the colony. Contrarily, there was a significant decrease of active bees in colony C (Figure 3). Research (Tasei *et al*., 2000) (Mommaerts *et al*., 2010) has found a decrease in reproductive output when exposed to neonicotinoids which could also explain lower active populations within the flight arenas. Mommaerts *et al.* (2010) also explained signs of apathy towards foraging in treatment colonies and could further explain lower numbers of active bees in our foraging arenas. Similarly, as with existing research on hyper to hypoactivity mentioned in Visitations, we see an immediate increase in active populations in colony C before the decline (Suchail, Guez and Belzunces, 2001) and fitting to mathematical model predictions (Bryden *et al*., 2013).

Deaths

Significant differences in mortality rates were found between all colonies (Figure 4). The lowest mortality rate was found in colony A and the highest in colony C. Existing research (Crall *et al*., 2018) has discovered detrimental behavior within the nests of bees subjected to neonicotinoids. General activity and nursing within the nests was found to be significantly lower as well as an increased distance from the centre of the nests; i.e. the bees were more spread out and less centralised as a colony. We predict this research sheds light on why colonies B and C suffered significantly higher mortality rates as a result of impaired natural behavior. Table 1 shows the total populations at the termination of the experiment and provides backing of lower reproductive behavior as there are clear differences between the treatment colonies and colony A. Total populations and deaths fit with current research and mathematical modelling (Bryden *et al*., 2013)

Further discussion

In order to start better managing wild pollinators for economic and conservational gain it is first important to understand the impacts that current practices have on them. We were able to suggest, fortified by existing research, that, from the onset of acute exposure to the pesticide, foraging behavior of *Bombus terrestris* was significantly negatively impacted. Extrapolating the results of this experiment, to a realistic setting, suggests that an increased foraging time through apathy or docility coupled with fewer overall visitations could have a substantial impact on both production crops and the wild ecosystems that are under the pollination management of the bumblebee. At this juncture it becomes vital to weigh up whether the benefits of using

imidacloprid are outweighed by the costs as issues such as these could mean that crop yields diminished could further broaden pollination deficits faced in current farming practices.

Critically, it would be important to apply this research to the field in order to ascertain pertinence to wild bee populations outside the lab. Arce *et al*., (2016) completed a similar field-based experiment and found contrasting results stating only minor foraging pattern differences. This previous report finds that the main significance was in the early stages of the experiment, as in the treatment colony foragers returned to their hives with pollen more regularly. Both colonies showed parabolic curves of increased activity followed by a decline; attributed to the colony aging. The other significance found in this study is that this rate of incline and decline was slower in treatment colonies. Other field based research is limited however and more critical analysis of this experiments data needs to be put to the test in field experiments.

Limitations and further work.
The time available to carry out this experiment limited the reliability. Time frames in similar research (Mommaerts *et al.,* 2010) were carried out over up to 40 weeks. Our small sample size did not allow for us to consider inherent extreme behavioral or physiological differences between the colonies. Arce *et al*., (2016) by contrast, replicated treatments in their experiment using 20 colonies.

It would be important to carry out this experiment in a field-realistic setting. Our results may have been buffered by the perfect conditions such as weather, light and food availability. Similarly, they may have been exacerbated by exclusively providing contaminated *nectar* intensifying their exposure and reaction to imidacloprid. Studies (Arce *et al.,* 2016) in field realistic settings have shown contrasting results to those gathered in this experiment.

Bibliography

Arce, A., David, T., Randall, E., Ramos Rodrigues, A., Colgan, T., Wurm, Y. and Gill, R. (2016). Impact of controlled neonicotinoid exposure on bumblebees in a realistic field setting. *Journal of Applied Ecology*, volume 54, issue 4, pages 1199-1208.

Bryden, J., Gill, R., Mitton, R., Raine, N. and Jansen, V. (2013). Chronic sublethal stress causes bee colony failure. *Ecology Letters*, volume 16, issue 12, pages 1463-1469.

Crall, J., Switzer, C., Oppenheimer, R., Ford Versypt, A., Dey, B., Brown, A., Eyster, M., Guérin, C., Pierce, N., Combes, S. and de Bivort, B. (2018). Neonicotinoid exposure disrupts bumblebee nest behavior, social networks, and thermoregulation. *Science*, volume 362, issue 6415, pages 683-686.

Degen, J., Kirbach, A., Reiter, L., Lehmann, K., Norton, P., Storms, M., Koblofsky, M., Winter, S., Georgieva, P., Nguyen, H., Chamkhi, H., Greggers, U. and Menzel, R. (2015). Exploratory behaviour of honeybees during orientation flights. *Animal Behaviour*, volume 102, pages 45-57.

Dively, G. and Kamel, A. (2012). Insecticide Residues in Pollen and Nectar of a Cucurbit Crop and Their Potential Exposure to Pollinators. *Journal of Agricultural and Food Chemistry*, volume 60, issue 18, pages 4449-4456.

Fauser-Misslin, A., Sadd, B., Neumann, P. and Sandrock, C. (2013). Influence of combined pesticide and parasite exposure on bumblebee colony traits in the laboratory. *Journal of Applied Ecology*, volume 51, issue 2, pages 450-459.

Gill, R. and Raine, N. (2014). Chronic impairment of bumblebee natural foraging behaviour induced by sublethal pesticide exposure. *Functional Ecology*, volume 28, issue 6, pages 1459-1471.

Goulson, Dave. (2003). Conserving wild bees for crop pollination. *International Journal of Food Agriculture and the Environment*, volume 1, issue 1, pages 1-2.

Julier, H. and Roulston, T. (2009). Wild Bee Abundance and Pollination Service in Cultivated Pumpkins: Farm Management, Nesting Behavior and Landscape Effects. *Journal of Economic Entomology*, volume 102, issue 2, pages 563-573.

Kevan, P., Clark, E. and Thomas, V. (1990). Insect pollinators and sustainable agriculture. *American Journal of Alternative Agriculture*, volume 5, issue 1, pages 13-22.

Laycock, I., Lenthall, K., Barratt, A. and Cresswell, J. (2012). Effects of imidacloprid, a neonicotinoid pesticide, on reproduction in worker bumble bees (Bombus terrestris). *Ecotoxicology*, volume 21, issue 7, pages 1937-1945.

Mommaerts, V., Reynders, S., Boulet, J., Besard, L., Sterk, G. and Smagghe, G. (2009). Risk assessment for side-effects of neonicotinoids against bumblebees with and without impairing foraging behavior. *Ecotoxicology*, volume 19, issue 1, pages 207-215.

Potts, S., Biesmeijer, J., Kremen, C., Neumann, P., Schweiger, O. and Kunin, W. (2010). Global pollinator declines: trends, impacts and drivers. *Trends in Ecology & Evolution*, volume 25, issue 6, pages 345-353.

Ricketts, T., Regetz, J., Steffan-Dewenter, I., Cunningham, S., Kremen, C., Bogdanski, A., Gemmill-Herren, B., Greenleaf, S., Klein, A., Mayfield, M., Morandin, L., Ochieng', A. and Viana, B. (2008). Landscape effects on crop pollination services: are there general patterns?. *Ecology Letters*, volume 11, issue 5, pages 499-515.

Suchail, S., Guez, D. and Belzunces, L. (2001). Discrepancy between acute and chronic toxicity induced by imidacloprid and its metabolites in *Apis mellifera*. *Environmental Toxicology and Chemistry*, volume 20, issue 11, pages 2482-2486.

Tasei, J., Lerin, J. and Ripault, G. (2000). Sub-lethal effects of imidacloprid on bumblebees,Bombus terrestris (Hymenoptera: Apidae), during a laboratory feeding test. *Pest Management Science*, volume 56, issue 9, pages 784-788.

Willmer, P., Bataw, A. and Hughes, J. (1994). The superiority of bumblebees to honeybees as pollinators: insect visits to raspberry flowers. *Ecological Entomology*, volume 19, issue 3, pages 271-284.

Wu-Smart, J. and Spivak, M. (2017). Effects of neonicotinoid imidacloprid exposure on bumble bee (Hymenoptera: Apidae) queen survival and nest initiation. *Environmental Entomology*, volume 47, issue 1, pp.55-62.

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