Title

Feeding behavior in queen bumble bees (Bombus impatiens) is not affected by the presence of imidacloprid in nectar

Author
Bratu, Jade

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FEEDING BEHAVIOR IN QUEEN BUMBLE BEES (BOMBUS IMPATIENS) IS NOT AFFECTED BY THE PRESENCE OF IMIDACLOPRID IN NECTAR

By

Jade Bratu

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Dr. S. Hollis Woodard
Department of Entomology

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Dr. Richard Cardullo, Howard H Hays Chair and Faculty Director, University Honors
Interim Vice Provost, Undergraduate Education

Abstract

Bees are sensitive to the content of the nectar they consume, and human activities are negatively affecting these contents. It is common practice to spray pesticides on crops; however, non-target plants have been found to contain trace amounts of these pesticides in nectar as a result, and this has a negative impact on wild bee populations. Imidacloprid-laced nectar was fed to a group of queen Bombus impatiens, and the amount of nectar consumed was measured and compared to control queens that had not been exposed to the imidacloprid. Overall, there was no significant difference in feeding behavior between the two groups. This finding suggests that bumble bees are unable to detect and thus avoid imidacloprid they are exposed to, thus rendering them vulnerable to the sub-lethal effects of the pesticide.

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Table of Contents

Introduction

Pollinator health has become a prominent concern in recent decades. The decrease in the number of pollinators has led to increased pressure on researchers to study the effects of pesticides on beneficial insects, such as bees (Kluser and Peduzzi, 2007**.** These insects are essential in the pollination of many species of plants, including crops. About a third of the American food supply comes from plants that require insect pollination, and if these plants are not pollinated, they cannot produce high quality food (Kluser and Peduzzi, 2007). Food security is already a problem; despite recent advances, 1 in 7 people still do not get enough energy out of their food (Godfray et al., 2010). In addition, agricultural land is in short supply, so land that is available for producing food needs to be as efficient as possible (Pretty, 2008). As pollinator health declines, so will food production, aggravating this problem.

Imidacloprid is a neonicotinoid insecticide that affects the central nervous system of insects. It binds to the receptors of insect nerve cells and disrupts the nerve's ability to send signals. Imidacloprid can be taken up by plants, and can then spread into the plant's tissues and products, including nectar and pollen (Gervais et al., 2010). This means that insects that consume these plants and their floral rewards, such as aphids, flies, and bees, will be exposed to imidacloprid, resulting in death at high enough doses (Gervais et al., 2010). At lower doses, the insecticide impacts individual behavior, increasing or decreasing food consumption, potentially influencing how much of the pesticide that the individual is exposed to (Kessler, S. C. et al., 2015). In bumble bees, sub-lethal doses of imidacloprid were also shown to impair worker foraging and homing abilities, meaning that they were unable to bring enough pollen back to the colony (Gill et al., 2012).

Imidacloprid can persist in the soil and water for years, so it can be taken up by plants that were not the intended target of the pesticide (Gervais et al., 2010). This is a problem for any pollinators of these wild plants, such as bumble bees. For example, imidacloprid has been shown to drastically reduce colony growth and queen production in the bumble bee *Bombus terrestris* (Whitehorn et al., 2012). A recent experiment showed that bees cannot only detect imidacloprid in their food, but may preferentially feed on it; both *Apis mellifera* and *Bombus terrestris* preferred nectar that contained imidacloprid at doses as low as 1nM, or 0.256 ppb (Kessler, S. C. et. al, 2015). This increase in feeding increases the pesticide dose the bees are exposed to, which can lead to some of the more severe symptoms associated with greater exposure to imidacloprid.

Queen bumble bees are of special importance in regard to colony production and population dynamics. A healthy queen can produce hundreds of workers and reproductive males and queens. If she is exposed to a sub-lethal dose of imidacloprid at a young age, her ovaries will not develop properly, and her ability to produce offspring and contribute to the next generation of bees will be severely diminished (Baron et al., 2017). If she is exposed to a lethal dose, an entire potential colony and generation of reproductives die with her.

To test whether or not imidacloprid affects an individual queen's feeding behavior, feeding trials were run on *Bombus impatiens* queens during the early nest-founding stage (prior to egg laying). The amount of nectar consumed was carefully measured in order to determine whether or not imidacloprid causes a change in feeding rate. This is a critical period in the development of a colony, and if a queen increases the amount of nectar she consumes and thus the dose of imidacloprid she is exposed to, it will greatly affect her fitness and the future colony's health (Baron et al., 2017).

Methods

32 young *Bombus impatiens* queens of unknown age were removed from their natal nests and placed into individual plastic containers (15 x 8 x 8 cm) and stored in an incubator constantly maintained at 28 C and 60 % RH. Queens were immediately gassed with CO2 for thirty minutes upon removal from their colonies. This was done for two consecutive days in order to cause them to bypass diapause and initiate the egg-laying stage (Roseler 1985). For a week after being subjected to the CO2 treatment, all queens were fed 50% sucrose solution (w/v) and pollen (mixed source from Brushy Mountain) *ad libitum.* A preliminary analysis revealed a significant increase in feeding after the CO2 treatment; queens consumed more than double the amount of nectar than they normally would (based on all collected data) in the 7 days following CO2 treatment. This could be due to the fact that the queens are starting to produce eggs and thus need more energy initially. To avoid this period of artificially-increased feeding, we waited a week before beginning to measure consumption. Queens were kept in darkness except when feeders were changed every other day. Seven days after removal from the colonies, the queens were moved to new plastic containers (12 x 13 x 8 cm) with four horizontally positioned, 2.5 mL feeder tubes, and the treatment and data collection began.

The imidacloprid treatment group queens (N = 16 per group) were treated continuously with 50% sucrose containing imidacloprid at 5 ppb and the untreated (control) queens remained on the original 50% sucrose solution. This concentration of imidacloprid was used because it is within the field realistic range. According to a survey done by Carreck and Ratnieks (2014), the 10 ppb and 6 ppb used in many other experiments represents a worst-case scenario rather than a realistic dose. Field imidacloprid levels are extremely variable, ranging between 0.5 to 5.7 ppb.

In a survey done by Stewart et al. (2014) in the mid-southern part of the United States, imidacloprid was found to be present on the majority of a variety of crops. Nearby non-target wildflowers were then found to contain trace amounts of imidacloprid, averaging 4.0 +/- 5.5 ppb (Stewart, 2014). 5 ppb satisfies the ranges found in both studies. Queens were treated for 7 days. During this time period, nectar tubes were weighed before going into the cage and after forty-eight hours in order to estimate the amount of nectar consumed by calculating the change in mass. These masses were then converted to volume based on the weight of 50% sucrose solution (1.1900 g/mL).

Individual queen data were averaged to form one mean per treatment group. A Student's t-test (two-tailed, unpaired) was performed in order to determine if the treated queens had a different feeding rate than the control queens.

Results

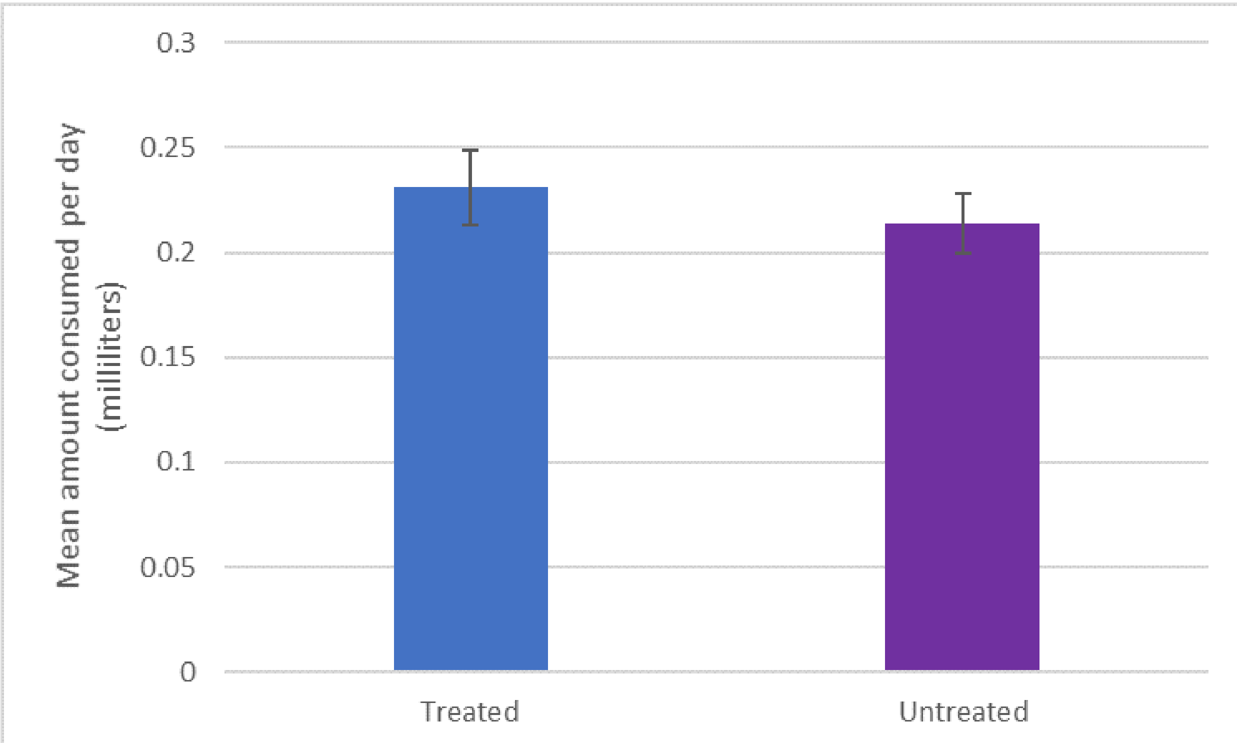

Figure 1. Bar graph demonstrating differences in the amount of nectar consumed between imidacloprid treated queens and non-treated queens; x-axis is the amount of nectar in milliliters consumed per day, the y-axis is the treatment group. Includes +/- standard error measurements. The difference was not significant.

The mean volume of nectar consumed per day by the treated queens was 0.231 mL (+/- 0.018 mL S.E.), with an average total of 3.698 mL (+/- 0.019 mL S.E.) consumed across the entire experimental period (7 days) for all treated queens. The mean volume of nectar consumed per day by the untreated queens was 0.214 mL (+/- 0.014 mL S.E.), with an average total of 3.425 mL (+/- 0.015 mL S.E.) consumed across the entire experimental period (7 days). There was no significant difference in the volume of nectar consumed between the two test groups ($P = 0.481$).

Discussion

In this experiment, bumble bee queens in the early nest-founding stage ate 0.223 mL of nectar per day, on average, and did not alter their feeding behavior (i.e., amount of nectar consumed) in the presence of a sub-lethal dose of imidacloprid. The finding that queens in this study did not feed differentially from imidacloprid-treated nectar may be explained by queens simply not being able to detect that imidacloprid was present in the nectar, similar to what was described in Kessler's study. Though the two species of bee used in Kessler's study (*Bombus terrestris* and *Apis mellifera*) showed a preference for treated nectar, a test was performed on gustatory neurons in the proboscis to confirm whether or not the bees could actually taste the imidacloprid in their nectar. These neurons did not respond to treated nectar differently than untreated nectar, leading to the conclusion that the bees were unable to taste the imidacloprid. Though such a test was not performed on our *Bombus impatiens* queens, this could explain the lack of adjustment in feeding behavior. The queens in our experiment did not preferentially feed from the imidacloprid-laced nectar, indicating that it is possible that the queens could not detect the imidacloprid at such a low concentration. Potentially, if doses were higher, there is a chance that the bees could detect and avoid the insecticide. If bees could not detect imidacloprid at concentrations as high as 256 ppb (as found in Kessler et al. 2015), then it is unsurprising that they could not detect it at 5 ppb in our study.

Alternatively, it is possible that the queens could detect the imidacloprid, but they simply did not adjust their feeding in response to the insecticide. This experiment did not offer the bees an alternative source of nectar, so the bees may have continued to feed normally to avoid starvation. In the wild, the queens would have a wide variety of plants to forage from, and thus have a chance to avoid plants with imidacloprid. For example, oilseed rape is one of the major targets of neonicotinoids in the United Kingdom, and it is an extremely abundant crop; despite this, it only comprised 13% of the pollen collected by honey bees (Carreck and Ratneiks, 2014). When given a wide range of food choices, the chance that a queen will be forced to feed on nectar with imidacloprid in it is lowered. If the queens in this experiment had been given a choice of nectar, it is possible that they may have lowered their exposure.

Whether the bees could detect the imidacloprid or not, these results are troubling in regards to wild queens. If a queen is unable to detect imidacloprid in her food and does not change her behavior in its presence, then she will be subject to the sub-lethal effects of imidacloprid, compromising her ability to start a colony. Poisoned queens produce fewer bees, meaning that fewer bees will be available to pollinate flowers and crops for the next season.Thus, these results have negative implications for food security. Humans rely heavily on bees for the pollination of crops (Kluser and Peduzzi, 2007). Without bees, crops will not produce as efficiently as they could, exacerbating the problem of worldwide hunger (Godfray et al., 2010).

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