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Hazard/Risk Assessment

Effects of the Neonicotinoid Acetamiprid in Pollen on *Bombus impatiens* Microcolony Development

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Abstract: Honey bees and other wild bee species including bumble bees have experienced population declines in recent decades. While many stressors are implicated in bee population declines, much attention has focused on neonicotinoid pesticides, which are widely used and known to be toxic to pollinators. One neonicotinoid, acetamiprid, has been studied very little in bumble bees, despite its use on bumble bee pollinated crops. Here we assessed the impacts of acetamiprid to the North American bumble bee *Bombus impatiens* using the microcolony model. We examined nest growth, development, and subsequent nest productivity as measured by drone production. We found that high concentrations of acetamiprid in pollen (4,520 µg/kg) significantly impacted nest growth and development, and ultimately reproduction (drone production). We found the no observable adverse effects level to be 45.2 µg/kg. Overall, acetamiprid has the potential to negatively impact reproductive endpoints for *B. impatiens.* However, effects occurred at concentrations substantially higher than expected environmental concentrations that would be achieved when following label rates. Further work is

required to assess the effects of this pesticide on *B. impatiens* via alternate routes of exposure and on queenright colonies.

Keywords: bumble bee, invertebrate toxicology, neonicotinoid, microcolony, pesticides, risk assessment

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INTRODUCTION

Pollination services provided by bees are critical for crop success and maximizing agricultural yields (Brown and Paxton 2009; Potts et al. 2010). Honey bees (*Apis mellifera*), a non-native pollinator to North America, provide much of the pollination services in agricultural settings, however, other bees such as bumble bees (*Bombus* sp) also make important contributions. The bumble bee *Bombus impatiens* is native to the eastern United States and is used for commercial pollination of lowbush blueberries (Drummond 2012; Stubbs and Drummond 2001) and greenhouse tomatoes (Morandin et al. 2001; Velthuis and van Doorn 2006), among other crops (Lozier et al. 2011). Due to their larger body size, pollination techniques, and foraging behaviors, bumble bees have certain advantages over honey bees for pollination (Drummond 2012; Goulson 2010; Willmer et al. 1994). For instance, bumble bees can pollinate deeper flowers, utilize buzz pollination, and forage in cooler and wetter conditions than honey bees (De Luca and Vallejo-Marin 2013; Gradish et al. 2012; Willmer et al. 1994). Populations of some

bumble bee species within North America and Europe have declined in recent decades (Cameron et al. 2011; Colla and Packer 2008; Grixti et al. 2009; Lozier et al. 2011).

Many factors likely contribute to the observed bee population declines including pesticide use (Blacquière et al. 2012; Goulson 2013; Goulson et al. 2015), parasites (Goulson et al. 2015; Meeus et al. 2011), pathogens (Brown and Paxton 2009), and habitat loss and loss of quality forage associated with agricultural intensification (Goulson et al. 2005; Wood et al. 2019). Pesticide exposure has also been implicated (Goulson et al. 2008), but the degree to which pesticides contribute to observed population declines is not fully understood (Kleijn et al. 2015). One group of pesticides implicated in bee population declines are neonicotinoids, which are neurotoxicants that target the insect nicotinic acetylcholine receptor (Tomizawa and Casida 2003). Globally, this class of pesticides has rapidly gained in popularity due to high efficacy across a range of pests and low mammalian toxicity (Jeschke and Nauen 2008; Simon-Delso et al. 2015).

There are two classes of neonicotinoids, the nitro-group containing compounds (imidacloprid, clothianidin, thiamethoxam, dinotefuran, nitenypyram) and the cyano-group containing compounds (acetamiprid and thiacloprid) (Blacquière et al. 2012). Research efforts have focused on the nitro-group containing chemicals (e.g., imidacloprid) due to their acute toxicity to honey bees (Suchail et al. 2000) and concerns regarding their negative impacts on pollinators lead the European Union to restrict their use (EU 2013). This ban may result in increased usage of the cyano-group containing neonicotinoids (acetamiprid and thiacloprid).

Acetamiprid has received relatively little research attention. While most neonicotinoids are applied as a seed-coating and are systemic pesticides, acetamiprid is applied via foliar spray. It is approved for use on a variety of bumble bee-pollinated crops and application often coincides with the flowering stage of the plants (Elbert et al. 2008; USEPA 2002). Therefore, bumble bees may be exposed to acetamiprid via contaminated nectar and/or pollen (Böhme et al. 2018; David et al. 2016; Mullin et al. 2010). Pollen, while not critical for adult survival, is necessary for larval growth and development due to its protein and lipid content (Plowright and Pendrel 1977; Ribeiro 1994; Sutcliffe and Plowright 1990). Thus, exposure via pollen is an important route to consider when examining reproduction-related endpoints.

In order to examine the effects of pesticide-contaminated pollen on bumble bee nest growth and development, we utilized the microcolony model. In this experimental format, bumble bee workers are confined without a true queen, whereupon they will organize and establish a false queen that lays unfertilized eggs (Free 1955). Due to the haplodiploid sex determination system in bumble bees, the unfertilized eggs develop into males (i.e., drones) (Gardner and Ross 2013). The workers build nest structures and tend to brood, which are behaviors that align with worker activities in a full-size queenright colony. Microcolonies provide a means by which colony-level impacts of pesticide exposure can be assessed (e.g., worker survival, colony growth, development, and reproduction) in a controllable and repeatable way within the laboratory (Camp et al. 2019; Klinger et al. 2019; Laycock et al. 2014; Laycock et al. 2012; Siviter et al. 2020).

While acute toxicity values have been determined with acetamiprid for *B. impatiens* (Baines et al. 2017), neither chronic exposures, exposures via pollen, nor

exposures assessing colony growth and development have been conducted. In this work, we assessed the toxicity of acetamiprid delivered in pollen to microcolonies of *B. impatiens*. We examined microcolony growth, development, and productivity over seven weeks in order to determine whether acetamiprid negatively impacted population-relevant endpoints. This work is the first reported assessment of chronic acetamiprid toxicity with *B. impatiens* and provides critical foundational knowledge about an understudied and commonly used neonicotinoid pesticide.

MATERIALS AND METHODS

Chemicals and reagents

Acetamiprid (purity 99.9%) was obtained from Sigma Aldrich (CASRN 135410-20-7; St. Louis, MO) and used for pollen exposures. Inverted syrup was made with sorbic acid (Amresco, Dallas, TX), citric acid anhydrous (Fisher Scientific, Hampton, NH), pure cane sugar (Domino Foods, Inc) and distilled water (Gibco, Gaithersburg, MD).

Bumble bee stock

Newly emerged (<24 hrs old) *B. impatiens* workers (Biobest®, Romulus, MI) were acclimated to laboratory conditions for 24 hrs prior to microcolony initiation. Randomly selected workers were provisioned with 50/50 inverted sugar syrup (50% glucose and 50% fructose) prepared in distilled water, containing 2.95 mM citric acid and 8.32 mM sorbic acid. Pollen paste (2.5 g pollen/1 mL 50/50 inverted syrup) was prepared from honey bee corbicular pollen (multifloral, collected 2015 from ornamental nurseries, Connecticut, USA) was also provided. Palynologic analysis of the pollen used in this

study will be published separately. Bees were maintained in an environmental chamber (Percival, Perry, IA) in continuous darkness at 50 ± 5% relative humidity and 25 ± 0.5° C during acclimation and for the duration of the experiment.

Microcolony initiation, exposures, and monitoring

To initiate microcolonies, workers were separated into groups of five bees, bees were weighed, and then transferred to microcolony chambers. Chambers were composed of a 12.7 cm diameter stainless-steel geology sieve (ASTM 10, SciOptic US, Katy, TX), an acrylic base plate with holes for ventilation, a clear acrylic top plate with ventilation perforations as well as additional larger openings for accessing the chamber and for a feeding syringe. The small clear lid for the top plate was also present (custom made, EPA, RTP). An image of the chamber can be seen in Klinger et al (2019). Upon initiation, microcolonies were provisioned with 3 g of clean pollen paste placed in the lid of a 35 mm x 10 mm disposable petri dish and fed syrup via a 20 mL syringe (Medi-Dose-EPS, Ivyland, PA) with the end capped in place and a 1/8-inch access hole drilled at the 2 mL mark. This prevented the formation of an air pocket within the syringe that would obstruct the bees from feeding. Five days after initiation, an additional 2 g of clean pollen paste was added directly to the nest initiation dish. Beginning seven days after initiation, acetamiprid exposures began via 2 g of pollen paste provided in a separate feeding dish. Serial dilutions of syrup containing acetamiprid were used to generate the treatment pollen paste (see pollen paste methods above). No carrier solvent was required. Degradation of acetamiprid in aqueous solutions is minimal (Lewis et al. 2016), but we do not know how stable acetamiprid is in the treatment pollen pastes. Acetamiprid nominal concentrations in pollen were 0.452, 4.52, 45.2, 452, and 4,520 µg/kg to assess a

broad range of exposure concentrations Pollen feeding dishes were renewed Monday, Wednesday and Friday. Control microcolonies were provided clean pollen paste for the duration of the experiment. Similarly, syrup was renewed every Monday, Wednesday, and Friday for the duration of the experiment. Syrup syringes, pollen dishes, and microcolonies were weighed on renewal days to track food consumption and colony weight. Microcolonies were monitored weekly for adult worker mortality, number of egg chambers, number of brood masses, number of pupal cells, and drone emergence. Photo documentation was obtained weekly on Wednesdays for one microcolony per treatment to visually track microcolony development (iPhone 6, Apple, Cupertino, CA). Eclosed drones were removed from the microcolonies upon emergence and weighed. Microcolonies were maintained for seven weeks and upon termination nests were necropsied to quantify the contents of the nest. Eight microcolonies were used for each experimental group and two evaporation controls were run in parallel in order to account for baseline evaporation of syrup and pollen paste.

Data processing and statistical analysis

Syrup and pollen consumption values were corrected for evaporation prior to analysis. Instances of negative consumption rates were omitted from analyses. On occasion, syrup syringes leaked, leading to inaccurate syrup consumption values. In these instances, the inaccurate value was omitted and replaced with the average syrup consumption for that treatment group and day. The three microcolonies that perished before the termination of the experiment (two microcolonies in 45.2 µg/kg, and one microcolony in 0.452 µg/kg) were omitted from analyses for which a complete data set was necessary. Estimates of acetamiprid consumption on a per bee basis were corrected

for mortality. Differences between the control and treatment groups were analyzed with one-way analysis of variance (ANOVA) and Dunn's multiple comparison test when variances were no different. When variances were significantly different according to the Brown-Forsythe or Bartlett's tests, a non-parametric test was used (Kruskal-Wallis with Dunnett's multiple comparisons test). Data are presented as mean ± standard deviation (SD) and statistical significance was defined as $p<0.05$. All statistical analyses were performed with GraphPad Prism® (v6; La Jolla, CA).

RESULTS

Microcolony Workers

Average starting worker weights were no different between treatment groups (data not shown). The global average worker weight was 189.5 ± 20.2 mg. Worker survival was monitored throughout the seven weeks and no differences were found in percent survival between the treatment groups (data not shown). Average survival across all treatments was 78.3% ± 24.7%. Based on visual inspection of the microcolonies, worker behavior was unaffected by exposure to any concentration of acetamiprid tested.

Microcolony growth and development

Microcolony development was documented photographically each week to visually track microcolony growth and development (Fig. 1). Images of the 4,520 µg/kg treatment group showed noticeably smaller nest structures, which is consistent with quantitative measures of nest reproductivity obtained throughout the study.

Microcolony observations were recorded weekly and the number of capped egg chambers, brood masses, and pupal cells were tracked. Nest development progressed in the expected trajectory, with capped egg chambers occurring early in nest development, followed by a peak in brood masses, and finally with the peak of pupal cells (Fig 2a-c). Capped egg chambers were evident for all exposure groups during week 1, whereupon they tapered to zero by week 3 (Fig. 2a). Brood masses were evident by week 2 and peaked for most groups during that same week. Pupal cell formation began during week 3 of the microcolony experiment, with all groups showing pupal cells by week 4. Notably, the highest concentration group had significantly reduced pupal cells during week 4, 5, and 6 (Kruskal-Wallis test with Dunn's multiple comparisons test, $H = 25.1$, 5 df, $p<0.05$, One-Way ANOVA with Dunnett's multiple comparisons test, $F=14.9$, 5 df, $p<0.05$, One-Way ANOVA with Dunnett's multiple comparisons test, $F=6.29$, 5 df, $p<0.05$, respectively, Fig. 2c).

Nest weights were also measured throughout the course of the experiment and average weight change on a weekly basis was calculated (Fig. 2d). Microcolonies showed a clear trend in weight gain and loss throughout the 7-week experiment. Initially, all microcolonies lost weight during week 1, then steadily gained weight from weeks 2-4. During weeks 5-7, microcolony weights either declined or had marginal minimal gains as drones began emerging. Nest weight was significantly lower in the highest exposure concentration during week 3 and 4 of the experiment (One-Way ANOVA with Dunnett's multiple comparisons test, $F=4.1$, 5 df, $p<0.05$, Kruskal-Wallis test with Dunn's multiple comparisons test, $H=18.9$, 5 df, $p<0.05$, respectively, Fig. 2d).

Upon termination of the experiment, microcolonies were necropsied to determine the contents of the remaining nest structures, including live versus dead larvae, pupae, and pre-emergence drones. The 4,520 µg/kg exposure group was the only group to have more dead larvae present than live larvae and did not have any pupae or drones within the nests at termination (data not shown).

Microcolony food consumption

Average syrup consumption was assessed on a weekly basis. The highest acetamiprid treatment group (4,520 µg/kg) consumed significantly more syrup compared to control during the first week of the microcolony, then significantly less syrup during weeks 4, 5, and 6 (One-Way ANOVA with Dunnett's multiple comparisons test, $F=18.6$, 5 df, $p<0.05$, One-Way ANOVA with Dunnett's multiple comparisons test, $F=12.1$, 5 df, $p<0.05$, One-Way ANOVA with Dunnett's multiple comparisons test, $F=6.60$, 5 df, $p<0.05$, respectively) (Figure 3a). Overall, the lowest weeks of syrup consumption were the 1st and 7th. When total syrup consumption by treatment was considered, the highest acetamiprid treatment consumed significantly less (One-Way ANOVA with Dunnett's multiple comparisons test, $F=9.02$, 5 df, $p<0.05$) (Fig 3c).

Pollen consumption followed a similar trend to syrup consumption in that the weekly analysis revealed that pollen consumption was significantly reduced at the highest exposure concentration for multiple weeks (week 3 One-Way ANOVA with Dunnett's multiple comparisons test, $F=1.12$, 5 df, $p<.0.05$; week 4 Kruskal-Wallis test with Dunn's multiple comparisons test, $H=26.4$, 5 df, $p<0.05$; week 5 One-Way ANOVA with Dunnett's multiple comparisons test, $F=6.39$, 5 df, $p<.0.05$; and week 6, Kruskal-Wallis

test with Dunn's multiple comparisons test, H=22.4, 5 df, $p<0.05$; Fig. 3b). In general, pollen consumption peaked during week 4 of the experiment (Fig. 3b). When total pollen consumption was considered, the highest acetamiprid exposure group consumed significantly less pollen over the course of the microcolony experiment (One-Way ANOVA with Dunnett's multiple comparisons test, F=13.51, 5 df, $p<0.05$, Fig. 3d).

The amount of acetamiprid consumed by the microcolonies was estimated for each treatment and for individual workers (Table 1). The 4,520 µg/kg treatment group consumed on average 14.9 ± 3.9 µg of acetamiprid throughout the course of the 6 weeks of contaminated pollen exposure. When consumption of acetamiprid by individual bees was estimated, workers in the 4,520 µg/kg treatment group nominally consumed 3.1 ± 0.67 µg of acetamiprid.

Drone Production

Control microcolonies produced their first drones in the fifth week of the experiment and produced an average of 24.3 ± 7.0 drones (Fig. 4a). For controls, drones began emerging on day 33 (33.0 ± 0.0), and emergence was significantly delayed for the highest exposure group (37.3 ± 2.5 days, One-Way ANOVA with Dunnett's multiple comparisons test, F=8.29, 5 df, $p<0.05$, data not shown). When total average drone production was compared, the highest concentration produced significantly fewer drones (Kruskal-Wallis with Dunn's post-hoc comparisons, H=22.5, 5 df, $p<0.05$, Fig. 4a). Comparison of drone weights revealed that the two highest concentrations (452 and 4,520 µg/kg) produced significantly smaller drones (Kruskal-Wallis with Dunn's post-hot comparisons, H=29.9, 5 df, $p<0.05$, Fig. 4b). Drone emergence by week was also

examined, and we found that drone emergence was significantly decreased as compared to control for the second highest concentration during week 5 (One-Way ANOVA with Dunnett's multiple comparisons test, $F= 7.73$, 5 df, $p<0.05$) and the highest concentration in both week 5, 6 and 7 One-Way ANOVA with Dunnett's multiple comparisons test, $F= 7.73$, 5 df, $p<0.05$, Kruskal-Wallis with Dunn's multiple comparisons test, $H=20.9$, 5 df, $p<0.05$, Kruskal-Wallis with Dunn's multiple comparisons test, $H=19.1$, 5 df, $p<0.05$; Fig. 4c).

DISCUSSION

Here we assessed the impact of chronic acetamiprid exposure via pollen on *B. impatiens* worker survival and microcolony growth and development. The microcolony model was developed more than two decades ago and has been successfully used for investigations of bumble bee behavior, gut microbiome, nutrition, development, pathogens, chemical biology, and pesticides (Reviewed in Klinger et al. 2019). Using this model, we found that the highest concentration assessed (4,520 µg/kg) resulted in adverse effects on colony growth (larval mortality), decreased food consumption (both pollen and syrup), and overall impaired nest productivity (reduced drone production and drone weights), however, worker survival was not affected. Treatment groups exposed to lower acetamiprid concentrations were unaffected, except for 452 µg/kg, which also reduced average drone weights. Thus, the no observable adverse effects level (NOAEL) for microcolony growth and development was found to be 45.2 µg/kg. Although NOAELs are routinely used by regulatory agencies, the utility of these values for ecotoxicology assessments has been questioned (Laskowski 1995). Recognizing that the dosing interval selected for this study was broad (i.e., 10-fold), conducting additional studies using more

microcolonies per exposure group, a narrower interval between concentrations, and repeating the study are needed to more reliably estimate the NOAEL.

Notably, *B. impatiens* workers were not adversely impacted by chronic exposure to acetamiprid in pollen at the concentrations tested here. Based on the limited data available, nonqueen bumble bee larvae and adult workers consume 10-40 mg/day and 20-30 mg/day, respectively (reviewed inGradish et al. 2019). While pollen consumption is not necessary for worker survival, and clean syrup was provided *ad libitum* as a carbohydrate source, the workers were still exposed to acetamiprid through various means. Pollen is both a source of protein and lipids for bumble bees, and workers will consume pollen in situations where male egg-laying occurs in order to obtain nutrients for ovary development (Vaudo et al. 2017). As such, at least one worker within each colony was consuming pollen for the purpose of egg-laying. However, it is possible that most of the false queen's pollen consumption occurred prior to the introduction of the contaminated pollen (i.e., during week 1). Furthermore, workers handle pollen in order to build nest structures and feed brood, which requires mastication and manipulation. Thus, it is likely that all workers within the microcolonies were exposed to acetamiprid, but limited information on pollen consumption rates and the multifunctional role of pollen in the microcolony makes it difficult to determine the exact amount of pollen directly consumed by individual workers and brood. Therefore, we calculated estimates of worker consumption but also the total amount of acetamiprid that the microcolonies utilized as a whole..

Within our experiment, several lines of evidence suggest that highest concentration of acetamiprid tested here (4,520 µg/kg) negatively impacted larval bumble

bee growth and development. We observed no reduction in the number of brood masses; however, the number of pupal cells was significantly reduced. This suggests that few larvae survived long enough to begin pupation. Further evidence of larval mortality was found in nest necropsy results, wherein the 4,520 µg/kg nests contained more dead larvae than live larvae, which was not observed for any other treatment group. Taken together, feeding *B. impatiens* brood acetamiprid-contaminated pollen at 4,520 µg/kg resulted in larval mortality and provides an explanation for the observed reductions in drone production. This observation has precedence within the literature, as acetamiprid can cause larval mortality in a variety of other invertebrates (Amirzade et al. 2014; Kuhar et al. 2006; Mo et al. 2002; Zabel et al. 2001).

Our assessments of pollen and syrup consumption showed that both food types were consumed at a lower rate in the highest acetamiprid concentration tested (4,520 µg/kg). This suggests that bumble bee workers were not avoiding the contaminated pollen due to palatability or aversion, but rather had a reduced need for resources. This is consistent with our observation of larval mortality, as reduced larval survival would consequently reduce nest food requirements. The lack of aversion to neonicotinoid-containing food observed here is consistent with other studies that found bumble bees did not avoid neonicotinoid-contaminated syrup or pollen (Gels et al. 2002; Larson et al. 2013). Another study demonstrated that bumble bees prefer neonicotinoid-containing foods (Kessler et al. 2015). While our results are generally aligned with these reports, none of these reports included cyno-group containing neonicotinoids like acetamiprid in their study design. For that reason, additional studies are necessary to better understand how bumble bees respond to foods containing acetamiprid.

There are multiple explanations as to why bumble bee larvae may be more sensitive to acetamiprid exposure than adult workers. One explanation may be differences in p450 enzyme expression and activity between the life stages. It is known that in both honey bees and bumble bees that CYP9Q subfamily of p450 enzymes can metabolize the cyano-group containing neonicotinoids, thiacloprid and acetamiprid (Manjon et al. 2018; Troczka 2019). This enzyme is likely responsible for the lower toxicity of acetamiprid as compared to the nitro-group containing neonicotinoids such as imidacloprid. However, it is unknown whether larval bumble bees express this enzyme and can detoxify acetamiprid. Further, since acetamiprid is a neurotoxicant, it is possible that larval bumble bees are more sensitive to nervous system perturbation. And finally, it is possible that on a per mass basis, larvae may have experienced a higher exposure. Future studies are needed to clarify the differences in larval versus adult toxicity to pesticides for bumble bees.

Field studies have confirmed that bees can be exposed to acetamiprid via pollen. In honey bee corbicular pollen collected in Germany from 2012-2016, acetamiprid was detected in 21 out of 281 samples and the highest concentration detected was 42.7 µg/kg, which is just under the maximum residue level in the European Union for acetamiprid of 50 µg/kg (Böhme et al. 2018). In another examination of corbicular pollen collected in Spain, acetamiprid was present at concentrations ranging from 7-104 µg/kg (Calatayud-Vernich et al. 2018). In a United States study surveying the presence of agricultural chemicals in honey bee pollen, acetamiprid was detected and the highest concentration was equivalent to 134 µg/kg (Mullin et al. 2010). These field concentrations, which are at least 33x lower than the concentration that elicited effects here, are unlikely to result in

adverse effects to developing bumble bees or workers when present in pollen and not combined with other toxicants. For comparison, acute toxicity occurs for *B. impatiens* at 250,000 µg/L (96hr LC_{50}) when administered orally, which is about 55x higher than the top concentration tested here (Baines et al. 2017).

There are no published studies investigating the effects of chronic exposure to acetamiprid on bumble bee microcolonies or queenright colonies limiting our ability to make comparisons between studies. However, there are four microcolony studies available that investigated the effects of *B. terrestris* microcolonies exposed through diet to neonicotinoids (Elston et al. 2013; Laycock et al. 2014; Laycock et al. 2012; Mommaerts et al. 2010; Smagghe et al. 2013). Due to differences in study design including bee species (all used *B. terrestris*), study duration (range: 2 – 11 weeks), test compound delivery (only the study by Elston et al. 2013 used pollen to deliver the test compound), and the limited number of neonicotinoids tested it is very difficult to make meaningful comparisons to our study. Focusing on the only two studies that investigated the effects of thiacloprid, a cyano-group containing neonicotinoid, the effective concentration to reduce drone production by 50% (EC_{50}) in *B. terrestris* microcolonies fed syrup containing thiacloprid for 11 weeks was 12 ppm; a value 1/10 of the maximum field recommended concentration and approximately three times lower than values reported for imidacloprid and thiamethoxam (Mommaerts et al. 2010).

We are only aware of one published study investigating the effects of a cyano-group containing neonicotinoid (i.e., thiacloprid) on queenright bumble bee colonies (Ellis et al. 2017). In this study, queenright *B. terrestris* colonies were allowed to free forage on thiacloprid-treated raspberries beginning 0 – 4 days after application of the

compound when applied according to the manufacturer's recommended spray rate. Compared to control colonies, thiacloprid exposed colonies were more likely to die prematurely, weighed less at study conclusion and produced fewer reproductives. These results suggest that thiacloprid may not be as safe for bees as previously believed. Furthermore, they underscore the need to conduct additional studies with acetamiprid to more fully develop its toxicity profile. The importance of drones to bumble bee colony success is only recently beginning to be appreciated (Belsky et al. 2020). Future investigations should include measures of drone reproductive fitness.

It is important to note that neonicotinoids show synergistic toxicity with certain fungicides that may be used simultaneously in agricultural settings. Thiamethoxam has been shown to have synergistic toxicity with myclobutanil (Iverson et al. 2019) and imazalil (Raimets et al. 2018) and clothianidin has been shown to act synergistically with propiconazole (Sgolastra et al. 2017). Thus, while chronic exposure to field-realistic concentrations of acetamiprid did not alter adult survival, microcolony development, or brood production, in combination with fungicides or other pesticides, acetamiprid may negatively affect growth and survival at a lower concentration. Synergism is a dose-dependent phenomenon and adverse effects on growth and survival will not only depend on the specific components of the mixture, but also on the relative concentrations of the mixture components. Considering the low degree of toxicity observed in this study, the possibility of synergism occuring between acetamiprid and fungicides when used at recommended application rates may be limited. However, given the potential adverse consequences of synergism on bumble bee colony growth and survival and the highly limited number of published investigations, further investigation is warranted.

Our data suggest that acetamiprid in pollen has low chronic toxicity to *B. impatiens* workers. However, at concentrations well above field-relevancy, acetamiprid has the potential to reduce microcolony growth and development as well as drone production, the indicator of nest productivity within the microcolony model. As the first published investigation of the chronic effects of acetamiprid on *B. impatiens* workers and nest development, this study provides critical data on a widely used pesticide for a North American species of bumble bee.

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FIGURE CAPTIONS

Fig. 1 Microcolony nest progression through seven weeks of development. Weekly nest development images from representative microcolonies for each treatment group.

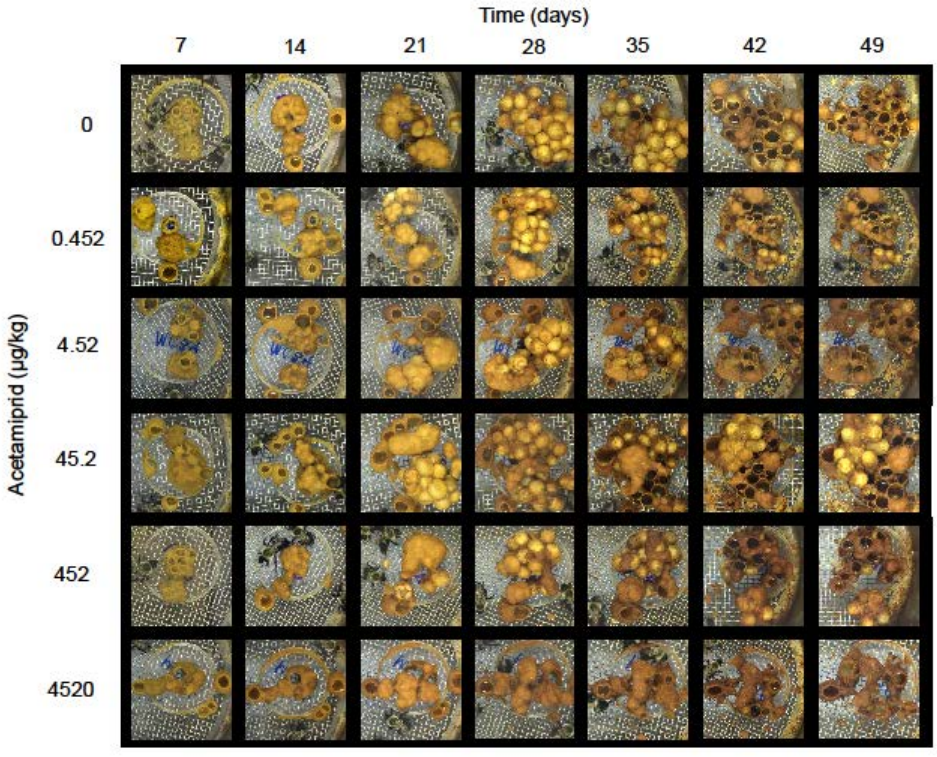

Fig. 2 Microcolony development. (a) Average capped egg chambers by exposure group per week. (b) Average brood masses by exposure group per week. (c) Average pupal cells by exposure group per week. (d) Average change in nest weight on a weekly basis. Data shown as mean ± SD. Asterisks denote statistical significance.

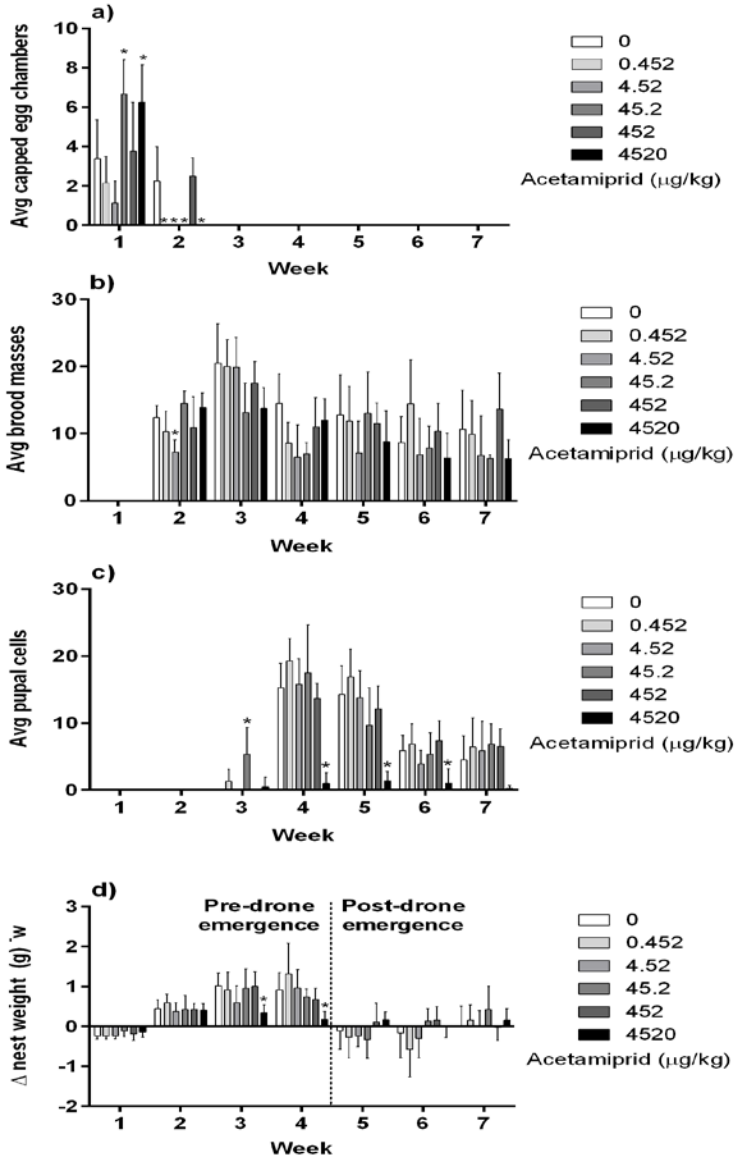

Fig. 3 Microcolony syrup and pollen consumption. (a) Average syrup consumption by exposure group per week. (b) Average pollen consumption by exposure group per week. (c) Average total syrup consumption over 7 weeks. (d) Average total pollen consumption over 6 weeks. Pollen consumption tracking began during week two of the experiment. All values have been corrected for evaporation. Data shown as mean ± SD. Asterisks denote statistical significance.

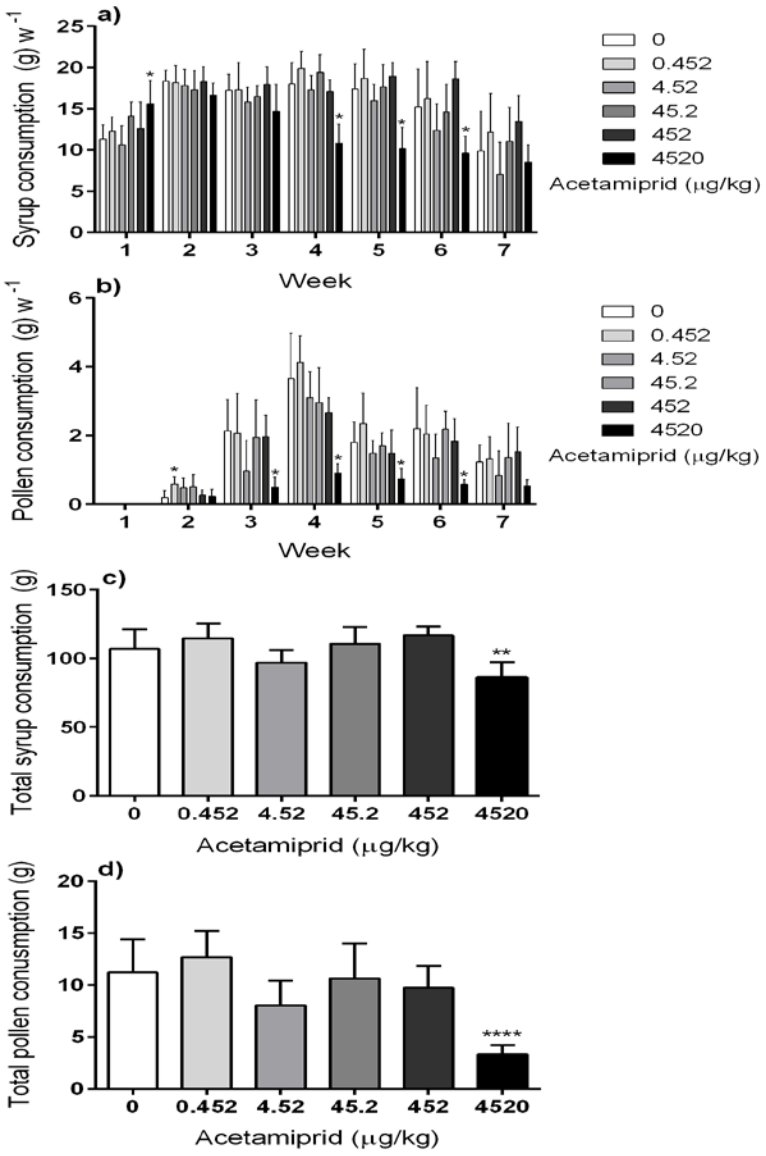

Fig. 4 Microcolony drone production. (a) Average total drones emerged by treatment group. (b) Average drone body weight by treatment group. (c) Average drone emergence by week by treatment group. Data shown as mean ± SD. Asterisks denote statistical significance.

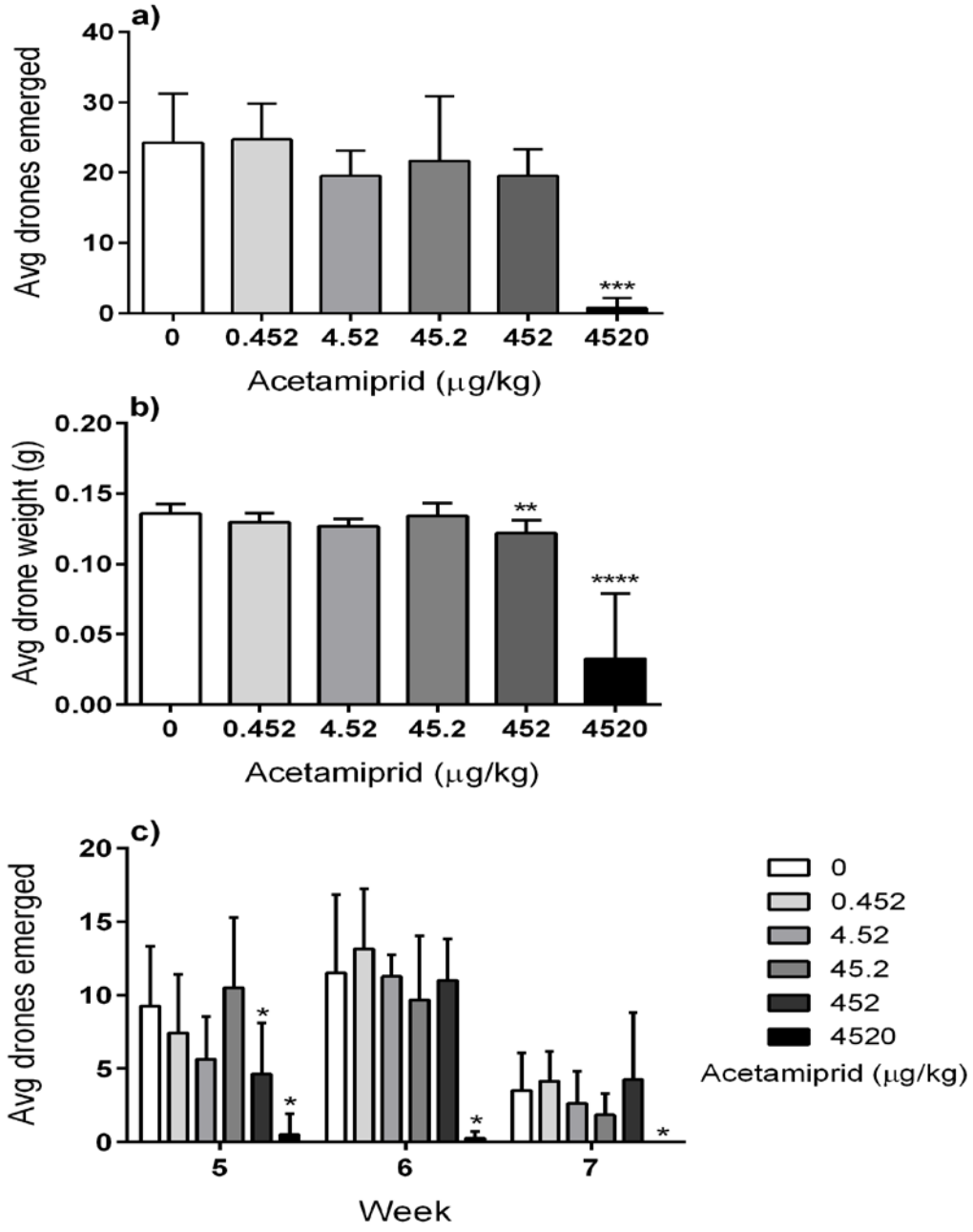

Table 1: Estimated acetamiprid utilized by whole microcolonies and individual workers.

Acetamiprid treatment (µg/kg)	0.452	4.52	45.2	452	4,520
Avg µg/MC	0.007 ± 0.001	0.048 ± 0.01	0.599 ± 0.15	5.59 ± 0.96	26.8 ± 3.9
Avg µg/bee[†]	0.0013 ± 0.0002	0.008 ± 0.002	0.1 ± 0.027	0.93 ± 0.23	3.14 ± 0.67

[†]Adjusted for worker mortality