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Effects of neonicotinoid imidacloprid exposure on bumble bee (Hymenoptera: Apidae) queen survival and nest initiation

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Abstract

Neonicotinoids are highly toxic to insects and may systemically translocate to nectar and pollen of plants where foraging bees may become exposed. Exposure to neonicotinoids can induce detrimental sublethal effects on individual and colonies of bees and may have long-term impacts, such as impaired foraging, reduced longevity, and reduced brood care or production. Less well-studied are the potential effects on queen bumble bees that may become exposed while foraging in the spring during colony initiation. This study assessed queen survival and nest founding in caged bumble bees [*Bombus impatiens* (Cresson) (Hymenoptera: Apidae)] after chronic (18-d) dietary exposure of imidacloprid in syrup (1, 5, 10, and 25 ppb) and pollen (0.3, 1.7, 3.3, and 8.3 ppb), paired respectively. Here we show some mortality in queens exposed at all doses even as low as 1 ppb, and, compared with untreated queens, significantly reduced survival of treated queens at the two highest doses. Queens that survived initial imidacloprid exposure commenced nest initiation; however, they exhibited dose-dependent delay in egg-laying and emergence of worker brood. Furthermore, imidacloprid treatment affected other parameters such as nest and queen weight. This study is the first to show direct impacts of imidacloprid at field-relevant levels on individual *B. impatiens* queen survival and nest founding, indicating that bumble bee queens are particularly sensitive to neonicotinoids when directly exposed. This study also helps focus pesticide risk mitigation efforts and highlights the importance of reducing exposure rates in the early spring when bumble bee queens, and other wild bees are foraging and initiating nests.

Key words: bumble bees, systemic insecticide exposure, nontarget risk

Numerous native plants and agricultural crops, including nutrient-rich fruits, nuts, and vegetables (Klein et al. 2007), are dependent on the pollination services provided by bees; however, bee populations are in decline across North America (NRCC 2007) including honey bees, bumble bees, and some solitary, specialist bees (Cameron et al. 2011). In addition to impacts on agricultural productivity and food security, pollinator decline can affect ecological balance when plant-pollinator networks become degraded and less resilient over time. Ecological disruption has been reflected by global losses in pollinator species richness, and temporal shifts in natural plant communities where parallel declines in insect-pollinated plants have been replaced with the greater abundance of wind-pollinated plants (Biesmeijer et al. 2006, Bartomeus et al. 2013, Burkle et al. 2013).

There are multiple factors that contribute to bee decline, alone or in combination, such as habitat loss, pests, pathogens, and pesticide exposure (Kremen et al. 2002, vanEngelsdorp et al. 2009, Potts et al. 2010, Bartomeus et al. 2013). Neonicotinoid insecticides, pervasively used in agricultural and urban areas, have been debated as one contributor to global bee losses (Godfray et al. 2014, 2015; van der Sluijs et al. 2015; Goulson 2015; Lundin et al. 2015; LaLone et al. 2017). Neonicotinoids are systemic and may translocate throughout the plant including the nectar and pollen where foraging bees may become unintentionally exposed. They are highly selective toward insects and exhibit low toxicity to mammals making this class of compounds safer for applicators compared with older classes of insecticides (Tomizawa and Casida 2003). Neonicotinoids target a broad spectrum of piercing and sucking insect pests by acting as nicotinic acetylcholine receptor (nAChR) agonists in the central nervous system (Tomizawa and Casida 2003, Elbert et al. 2008). Binding to nAChRs impairs normal cognitive and a suite of behavioral functions in bees, including learning, memory and other associated foraging tasks, mating, and nesting ability (Decourtye et al. 2003; Mommaerts et al. 2010; Feltham et al. 2014; Rundlöf et al. 2015; Stanley et al. 2015a,b; Wu-Smart and Spivak 2016).

Effects of neonicotinoids on worker bumble bees have been extensively studied (Mommaerts et al. 2010, Laycock et al. 2012,

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Laycock and Cresswell 2013, Feltham et al. 2014, Goulson 2015, Lundin et al. 2015, Rundlöf et al. 2015). Some studies have focused on the reproductive potential of worker bees in queenless colonies, and worker fecundity in queenright colonies (Laycock et al. 2012, 2013; Whitehorn et al. 2012); however, little has been researched on the direct impacts on queen bumble bees. More recent work has shown adverse effects on hibernation success, ovary development, and fecundity in *Bombus terrestris* (L.) (Hymenoptera: Apidae) queens exposed to the neonicotinoid, thiamethoxam (Baron et al. 2017, Fauser et al. 2017). Baron et al. (2017) also showed species-specific responses to thiamethoxam consumption and toxicity in four different bumble bee species (*Bombus terrestris*, *Bombus lucorum* (L.), *Bombus pratorum* (L.), and *Bombus pascuorum* (L.) (Hymenoptera: Apidae)), which highlights the need for more information about the potential impacts on other bumble bee species. These data also reveal one disadvantage of using only worker bees to estimate risk in nontarget assessments as queen bees may become exposed and affected differently than workers, in ways that lead to colony-level effects. In the spring, queens emerge from solitary hibernation, initiate a nest, and forage on their own until the first clutches of brood develop into workers and take on brood care and foraging roles (Sladen 1912, Free and Butler 1959, Evans et al. 2007). Queen bumble bees risk direct exposure to neonicotinoid-contaminated nectar and pollen when they forage to feed the first brood. Here we chronically exposed bumble bee queens to neonicotinoid (imidacloprid) in both syrup (0, 1, 5, 10, and 25 ppb) and pollen (0, 0.3, 1.7, 3.3, and 8.3 ppb), paired respectively, to examine the potential adverse effects on the queen bee during the solitary foraging and nest initiation phase. This study is the first to examine direct effects on *Bombus impatiens* bumble bee queens and provides greater insight to subsequent individual- and colony-level effects after exposure.

Materials and Methods

Experimental Set-Up and Treatments

Newly mated bumble bee queens (*Bombus impatiens* (Cresson) (Hymenoptera: Apidae)) were purchased in three batches from Koppert Biological Systems, INC Howell, MI in July and September 2014 and April 2015. The company ensured that all queens were of similar age and were in diapause for 5–7 months at the time of purchase. Carbon dioxide narcosis and cold treatments have been shown to shorten time to initial oviposition and increase oviposition rate after diapause in *B. ignites and B. terrestris* (Yoon et al. 2014). Therefore, to break diapause, queens were treated with carbon dioxide (99%) for 2 min then kept in cold storage (2°C) overnight. The following day all queens were treated again with carbon dioxide for 20 min then placed into narrow wooden nesting cages (5.7 × 13.4 × 11.0 cm) with side glass panels. The cages contained two chambers, the brood chamber and the foraging chamber. The brood chamber, where the nests were formed, was covered on the outside with a black plastic liner to maintain darkness and mimic underground nesting conditions. The foraging chamber was left uncovered allowing light to enter, simulating above ground foraging. Cages were held in a Percival incubator (model I-30NL) with a constant temperature of 30°C and relative humidity of 60–70%.

Queens from each purchase batch were randomly assigned to a treatment group, and 10 queens per treatment group were chronically exposed to imidacloprid in 50% sugar syrup at 0, 1, 5, 10, or 25 ppb for 18 d (Table 1). Between July 2014 and April 2015, a total of 30 queens were exposed at each concentration. These treatment levels and duration reflect a typical flower bloom period during which bees may become exposed to neonicotinoids when foraging on contaminated nectar and pollen in the environment. Stock solutions of imidacloprid (100 ppm) were prepared using 99.5 ± 0.5% technical grade imidacloprid purchased from Chem Service, Inc., West Chester, PA (PS-2086) dissolved with agitation in 50% sucrose overnight. Treatment solutions were prepared by making serial dilutions from the stock solutions every week. Treatment syrup (3 ml) was provided in small feeder cups in the foraging chamber three times a week, totaling eight feedings over 18 d. In addition, imidacloprid treatment syrup was mixed with honey bee-collected pollen and formed into pollen balls each weighing 2 ± 0.01 g (2:1 pollen to syrup). Final imidacloprid concentrations in the pollen ball provisions were 0, 0.3, 1.7, 3.3, and 8.3 ppb. Pollen balls were lightly coated with natural beeswax to maintain moisture and placed in the brood chamber. One treated pollen ball was given to each queen and remained in the cage for the duration of the experiment. The queens secreted wax to form a series of cells that comprise their nests on top of the provisioned pollen. The initial treated pollen ball remained in the cage as the nest was constructed on top. Subsequent untreated pollen balls were placed adjacent to and became incorporated with the core nest structure. Developing brood that fed on imidacloprid-contaminated pollen provisions were examined for potential impacts on brood survival and/or adult emergence. Control queens were given untreated syrup and pollen, and treated queens were given the following doses: 1 ppb syrup + 0.3 ppb pollen, 5 ppb syrup + 1.7 ppb pollen, 10 ppb syrup + 3.3 ppb pollen, or 25 ppb syrup + 8.3 ppb pollen (Table 2). Herein, the doses will be referred to by the syrup treatment levels (1, 5, 10, and 25 ppb) because this represents the exposure that treated queens experienced. After the 18-d treatment period, treated syrup was removed, and queens were given untreated syrup *ad libitum* and untreated pollen balls (2 g) every 2 wk.

The third set of queens purchased from Koppert on (April 2015) was used only for chemical residue analysis. These queens (10 per treatment group) were fed through the 18-d chronic treatment period then collected and frozen at −20°C.

Measurements

During the chronic exposure period, the volume of imidacloprid treatment syrup that remained after each feeding was measured to calculate the amount of syrup consumed. Potential effects of imidacloprid exposure on individual queen bees were determined every 48 h by recording queen mortality, and by time of nest initiation as defined by the presence of the first eggs. Effects on colony development were examined by quantifying brood production, the number of nectar pots (cup-like structures made from wax containing stored bee-collected syrup), and amount of dead larvae contained within or ejected from the nests during and after imidacloprid exposure. The experiment and measurements continued until the first clutch

Table 1. Average (± SD) syrup consumption (ml) in queens fed 0, 1, 5, 10, or 25 ppb imidacloprid (IMD) treatment before (Pre) and after (Post) nest initiation began

Syrup treatment (ppb)	Pre		Post	
	Ave	±SD	Ave	±SD
0	1.7	0.4	2.3	0.1
1	1.6	0.4	1.8	0.3
5	1.6	0.3	1.7	0.2
10	1.5	0.3	1.5	0.1
25	1.6	0.3	1.4	0.0

Pre nest initiation includes feedings 1–4 and post nest initiation includes feedings 5–7.

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of brood emerged as adults, marking the end of the queen's solitary phase and the end of the experiment. Due to logistic and facility constraints, we were not able to measure the impacts of exposure on emerging brood; however, after the first brood emergence, the nests were weighed and the remaining brood clusters were dissected to quantify the number of viable eggs, developing healthy larvae, and dead or discolored larvae and pupae (Fig. 1). Any surviving queens remaining after a total of 13 wk were terminated and nests were weighed and dissected.

Chemical Residue Analyses

Imidacloprid treatment syrup and pollen balls were tested for residues of imidacloprid and two metabolites, imidacloprid olefin and 5-OH imidacloprid. Samples were analyzed by USDA Agricultural Marketing Service, National Science Laboratory (AMS-NSL) in Gastonia NC. A total of 10 samples (3 ml) of imidacloprid treatment syrup (two per treatment) were collected from each replicate experiment. Treated pollen balls from replicate experiments were grouped together into one sample per treatment concentration (6–8 g) for chemical analysis. A total of 15 queen samples (three per treatment) from the final trial were also collected after the 18-d exposure period to confirm exposure rates due to imidacloprid treatments. Given the average weight of individual queens (<1 g) each residue sample consisted of three to four queens of the same treatment per sample to obtain enough material (>3 g) for more precise extraction and analysis. The USDA-AMS-NSL laboratory extracted samples using the QuEChERS (Quick, Easy, Cheap, Effective, Rugged, and Safe) pesticide extraction method (AOAC OMA 2007.01) and acetonitrile water solutions to test against certified standard reference materials. The samples were analyzed with liquid chromatograph- mass spectroscopy (LC–MS/MS) utilizing the parent and confirmatory ions of (imidacloprid, olefin, and 5-OH imidacloprid) analytes of interest.

Statistical Analysis

Survival analyses on the following measures, time to queen death, time to nest initiation (as indicated by the presence of eggs), and time to first brood emergence, were conducted using Kaplan–Meier curves and nonparametric analysis of covariance using Cox's Proportional Hazards model to test for differences among treatments compared with untreated queens over 13 wk (Kaplan and Meier 1958, Cox 1972). To test normality, Shapiro–Wilk's tests and visual comparisons of the data were made using quantile–quantile plots (Q–Q plot) and normal probability plots of the standardized data compared against the standard normal distribution. Data that did not fit a normal distribution were transformed using square-root, log10, or power^3 transformations as needed. To compare the effects across and among different treatment levels, one-way analysis of variance (ANOVA) modeling also was performed on the overall average number of days to queen death (square-root transformed), nest initiation (log10 transformed), and first brood emergence to account for censored subjects (i.e., queens that died or produced no brood) (Kuehl 2000). Nest weight was calculated by weighing each nest with all brood and dividing by the total grams of treated (one ball) and untreated pollen balls (one to six balls provisioned to each queen every 2 wk throughout the experimental period) because pollen balls are incorporated into the nest when

Table 2. The initial number of queens (*n*) compared with the number of queens that died, or that initiated nests and had brood that successfully emerged after 18 d of chronic imidacloprid treatment (ppb) in syrup (left)

Syrup treatment (ppb)	Pollen treatment (ppb)	*n*	Dead queens	Initiated nests	Emerged brood	Imidacloprid (IMD) residues (ppb)		
						Syrup	Pollen	Queens
0	0	21	3	17	17	ND	ND	ND
1	0.3	20	7	13	12	ND	ND	ND
5	1.7	20	6	14	12	4.8 ± 0.4	ND	1.9 ± 0.5
10	3.3	20	8	12	11	8.9 ± 1.6	2.2	2.9 ± 0.9
25	8.3	20	13	7	6	25.9 ± 2.3	7.1	7.1 ± 1.3

One untreated queen did not initiate a nest. One treated pollen ball was provided only at the initiation of the 18-d period to each colony, and remained in the colony; additional untreated pollen balls were provided every 2 wk after and were also incorporated into the nest structure. Chemical analysis of imidacloprid residues (± SD) in treatment syrup and pollen fed to queens, and all queens were collected at the end of the 18-d exposure period. Olefin and 5 OH-imidacloprid metabolites were not detected. Limit of detections for imidacloprid, olefin, and 5 OH-imidacloprid were 1, 10, and 25 ppb, respectively (right).

Fig. 1. Post experiment colony-level measures included final weight of nests containing brood clusters and nectar pots (left), quantification of eggs per cell (middle), larvae (right), and pupae (not shown).

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eggs are deposited and larvae develop directly in the pollen balls. There were no statistical differences between the two replicates (batches) within a treatment group; therefore, data from the 20 queens per treatment were pooled for all statistical tests. The rate of imidacloprid exposure via syrup consumption among treatments was divided analyzed separately for the first 9 d (feedings 1–4) and last 9 d (feedings 5–7, consumption not measured after feeding 8 due to collection error). The reason for discretizing the time variable was to examine syrup consumption rate among treatments before signs of nest construction were visible (feedings 1–4), as indicated by small pits formed in the provisioned pollen to deposit eggs, and after queens started nest construction (feedings 5–7), because there might have been differences in syrup consumption due to higher energy expenditures when queens began laying eggs. Differences among treatments in imidacloprid syrup consumption rate, nest weight, final queen weight (power^3 transformed), total brood count, and number of nectar pots were calculated using ANOVA and Tukey honest significant difference (HSD) multiple comparison mean separation tests at a significance of α = 0.05 to compare effects across and among different treatment levels. All statistical analyses were completed in Rstudio (version 3.2.0).

Results

Treatment Dosage

Imidacloprid syrup consumption prior to nest construction averaged (± SD) 1.6 ± 0.3 ml every 48 h and was not statistically different among treatment groups ($F_{4,490}$ = 6.2; P > 0.63) during the first 9 d of exposure (Table 1). After nest construction began, the average (± SD) syrup consumption by untreated queens was significantly greater (2.3 ± 0.1 ml), compared with consumption by treated queens at any of the tested concentrations (1.8 ± 0.6 to 1.4 ± 0.6 ml) ($F_{4,490}$ = 6.2; P < 0.0001). There were no significant differences in consumption rate by queens within each treatment level (P > 0.68) except by control queens (P < 0.0001). Chemical residue analysis of the imidacloprid syrup fed to queens and pollen provisioned for brood resulted in treatment levels very close to the intended concentration (Table 2). Imidacloprid residues were not detected in untreated and low-dose treatments of syrup (1 ppb) and pollen (0.3 and 1.7 ppb) and olefin or 5-OH imidacloprid residues were not detected in any sample, likely because the limit of detection for imidacloprid, olefin, and 5-OH imidacloprid was 1, 10, and 25 ppb, respectively. Imidacloprid residues in queens were detected in 5, 10, and 25 ppb syrup treatments at 28–38% of the treatment dosage (Table 2).

Queen Mortality

Mortality was observed in 14, 35, 30, 40, and 65% of queen bees fed 0, 1, 5, 10, and 25 ppb imidacloprid syrup, respectively (Table 1). Survival analyses indicated that queens treated at 1, 10, and 25 ppb syrup exhibited greater mortality, and death occurred significantly sooner, ($F_{4,33}$ = 3.95; P = 0.01) compared to untreated queens (P < 0.05), but not in queens treated at 5 ppb (P = 0.407) (Fig. 2).

Nest Initiation and Brood Emergence

Nest construction and initiation of egg-laying by untreated queens began on day 15 ± 2, significantly earlier than all treated queens that began laying eggs between days 23 ± 5 and 45 ± 17 ($F_{4,65}$ = 27.82; P < 0.0001) (Fig. 3a and c). All surviving untreated and treated queens eventually initiated nests; however, not all nests with eggs led to successful brood emergence. The experiment was terminated when remaining nests no longer showed overt signs of normal brood development or new brood clusters. Termination of all replicates occurred 13 wk after the queens were first brought out of diapause. At termination, there were five treated queens remaining, all of which had initiated nests but did not have emerged brood (Table 1). Dissection of these nests revealed either no healthy brood or brood at the egg and/or larval stages but no pupae, indicating potential effects on larval development. First brood emergence, or the curve marking the first worker bee to emerge as an adult, was significantly faster among nests established by untreated (control) queens, and nests established by queens exposed to 5, 10, and 25 ppb syrup treated queens ($F_{4,53}$ = 5.49; P < 0.0001). Time to first brood emergence in nests of 1 ppb treated queens was not different from untreated or other treated queens. The mean (± SD) number of days to first brood emergence was 50 ± 7, 57 ± 11, 60 ± 6, 60 ± 4, and 65 ± 10 in nests initiated by 0, 1, 5, 10, and 25 ppb syrup treated queens, respectively (Fig. 3b and d).

Colony-Level Measures

Colony-level measures were taken at the onset of first brood emergence, marking the end of the solitary phase and the end of the experiment for each individual queen that was able to establish a nest. There were no statistical differences among treatment groups in

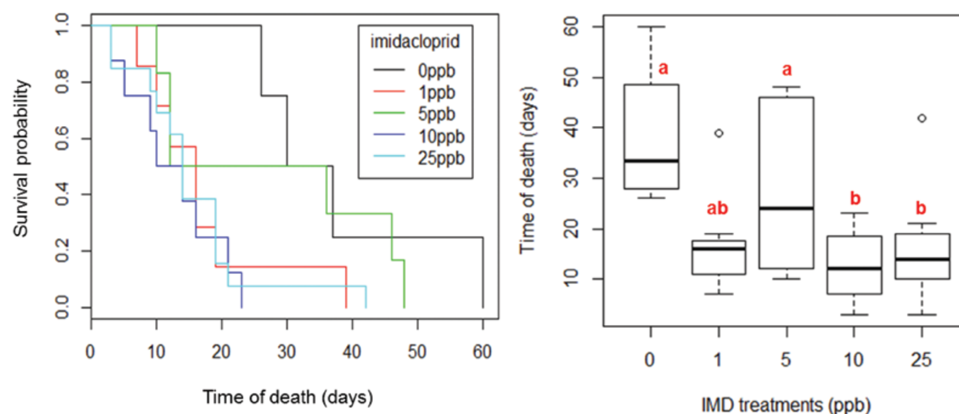

Fig. 2. Survival curve for queens chronically exposed to varying concentrations of imidacloprid (IMD) in syrup (0, 1, 5, 10, and 25 ppb) for 18 d (left). Box and whisker plots represent the median (black line) ± lower and upper interquartiles (box) and the minimum and maximum (whiskers) for the time of death among treatments (F=3.95; df = 4,33; P = 0.01) (right). Outliers are shown as open circles and different letters denote significance at α = 0.05. Results indicate significant differences between untreated queens and queens treated at 10 and 25 ppb.

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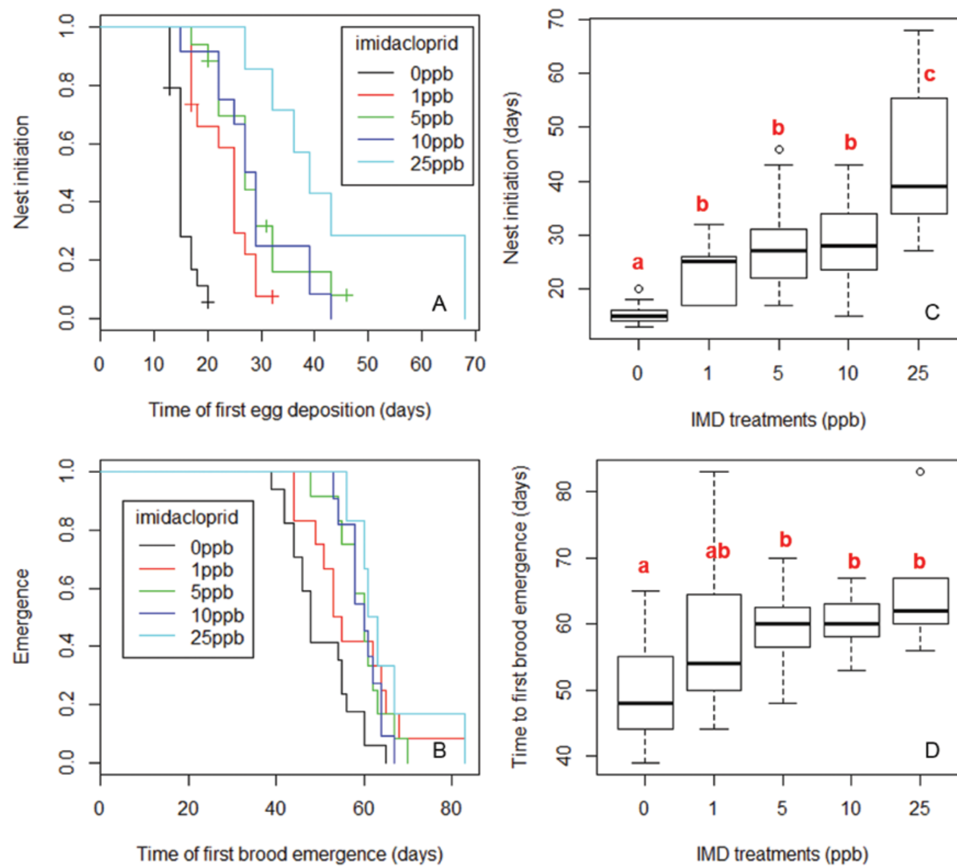

Fig. 3. Event curves for egg deposition, the indicator of nest initiation (F = 27.82; df = 4,65; P = 1.8e-13) (A), and first worker brood emergence (F = 5.49; df = 4,53; P = 8.9e-4) (B). The + at beginning of event steps correspond to nest initiation events by multiple queens observed on the same day. Box and whisker plots represent the median (black line) ± lower and upper interquartiles (box) and the minimum and maximum (whiskers) for the number of days to nest initiation (C) and first worker brood emergence (D) for queens treated with different imidacloprid (IMD) syrup treatments (ppb). Outliers are shown as open circles and different letters denotes significance at α = 0.05. Results show a dose-dependent effect on nest initiation and significant differences were observed in worker brood emergence between control and higher treatments (5, 10, and 25 ppb), but not at the lowest treatment (1 ppb).

the number of nectar pots ($F_{4,55}$ = 0.28; P = 0.89) total brood (eggs, larvae, and pupae ($F_{4,61}$ = 0.96; P = 0.44), and the number of dead or discolored larvae and pupae either ejected from or still contained within the nest ($F_{4,96}$ = 1.69; P = 0.16) (Fig. 4a and c) The proportional nest weight per gram of provisioned pollen given to queens was higher for untreated queens, with mean (± SD) 0.74 ± 0.1 compared with 0.67 ± 0.2, 0.59 ± 0.13, 0.59 ± 0.1, and 0.59 ± 0.1 g for 1, 5, 10, and 25 ppb syrup treated queens (Fig. 4b). There were significant differences in nest weight among untreated and 5 ppb and 10 ppb syrup treated queens ($F_{4,61}$ = 3.43; P = 0.01), but not between untreated and 25 ppb treated queens, likely due to low sample size of remaining 25 ppb treated queens (n=7).

Final queen weights (± SD) for untreated and 5 ppb treated queens (0.78 ± 0.1 and 0.76 ± 0.1 g, respectively) were significantly higher compared with queens in the highest 25 ppb syrup treatment (0.61 ± 0.1 g) ($F_{4,93}$ = 5.93; P = 0.0003) (Fig. 4d). There were no statistical differences in queen weights between 1 ppb (0.69 ± 0.1 g) and 10 ppb (0.72 ± 0.1 g) syrup treated queens when compared with all other treated and untreated queens.

Discussion

Our data indicate that bumble bee queens exposed to environmentally relevant levels of imidacloprid during the solitary nest founding phase of their life cycle may suffer reduced survival or delays in nest initiation, which in turn could negatively affect colony development and reproductive success of annual bumble bee colonies. During the nest initiation phase, queens may become unintentionally exposed to neonicotinoids when foraging on early spring blooming trees and wildflowers that may be contaminated from off-target drift of neonicotinoid seed-treated crops, such as corn, soy, and canola (Krupke et al. 2012, Botías et al. 2015, David et al. 2016, Long and Krupke 2016, 2017, Tsvetkov et al. 2017). Our survival analyses indicated a biphasic response on queen toxicity; significantly higher mortality was observed in the lowest and higher doses (1, 10, and 25 ppb syrup) compared with untreated and 5 ppb syrup treated queens (Fig. 2). This biphasic mortality response may be the result of multiple metabolic pathways activated by imidacloprid, or from action by toxic metabolites, and is consistent with other toxicity studies on neonicotinoids and bees (Suchail et al. 2000, Retschnig et al. 2014). Despite this biphasic response, the number of treated queens that died and the average time to death among treatments were similar (Table 2), indicating that the imidacloprid exposure at all field-relevant levels tested can be lethal to bumble bee queens. Nest initiation occurred over significantly narrow time frames; initial egg deposition occurred between days 13 and 20 in all untreated queens, including the three untreated queens that subsequently died for unknown reasons. In contrast, treated queens slowly continued to initiate nests for several weeks after exposure, which suggests a possible dose-dependent delay and recovery by queens once exposure

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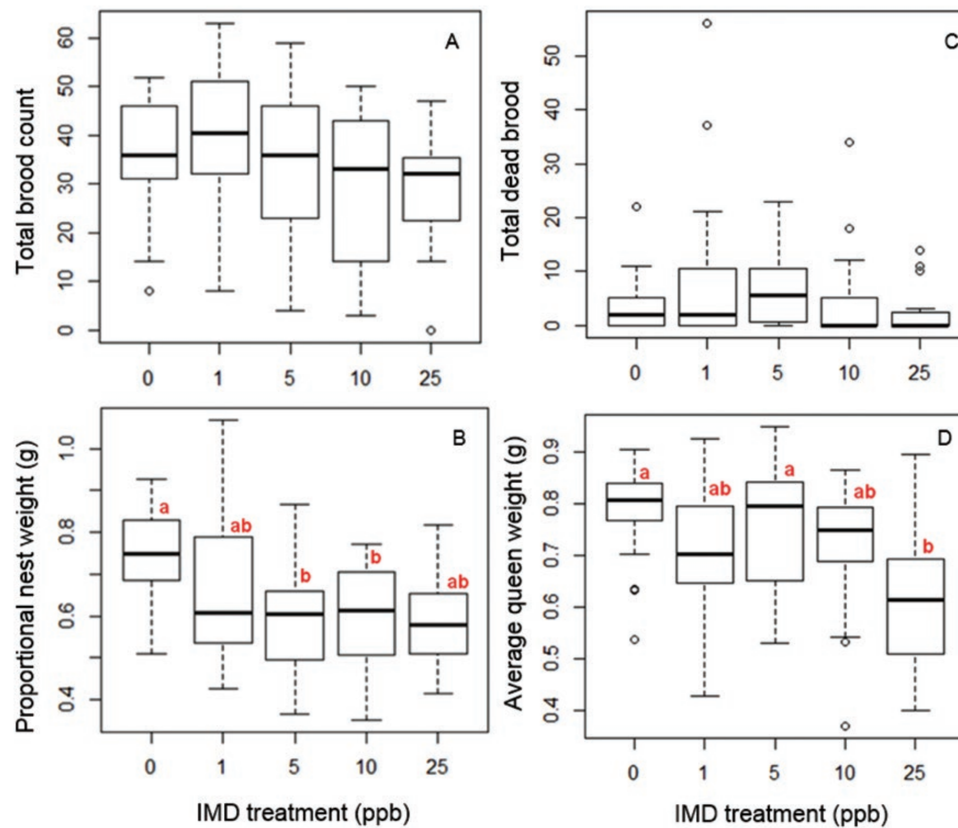

Fig. 4. Box and whisker plots represent the median (black line) ± lower and upper interquartiles (box) and the minimum and maximum (whiskers) for the total amount of healthy brood ($F = 0.96$; df = 4,61; $P = 0.44$) (A), the proportional nest weight per gram of provisioned pollen ($F = 3.43$; df = 4,61; $P = 0.01$) (B), dead or discolored brood (eggs, larvae, and pupae) ($F = 1.69$; df = 4, 96; $P = 0.16$) (C), and queen weight ($F = 5.93$; df = 4,93; $P = 0.0003$) (D) among nests in which queens were treated with different levels of imidacloprid (IMD) treatments (ppb). Outliers are shown as open circles and different letters denotes significance at $\alpha = 0.05$. No significant differences were found in total and dead brood measures. Differences were observed in final nest weight (B) and queen weight (D) but not in a dose-dependent manner.

ended. Delayed nest building activity has been shown in queenless microcolonies of *B. terrestris* worker bees exposed to thiamethoxam at 10 ppb but not at 1 ppb (Elston et al. 2013), indicating queen bees may be more sensitive to neonicotinoid toxicity compared with workers. Dose-dependent recovery from neonicotinoid exposure also has been shown using microcolonies of *B. terrestris* worker bees treated at varying imidacloprid doses ranging from 0.06 to 98 ppb in syrup (Laycock et al. 2013). Species-specific nesting behavior was observed in wild caught bumble bee queens (*B. terrestris*, *B. lucorum*, *B. pratorum*, and *B. pascuorum*) after chronic exposure to thiamethoxam (Baron et al. 2017), emphasizing the need to examine the effects of neonicotinoids on different species to more accurately assess overall risk to bumble bees.

The lack of differences in the amount of total brood produced by queens that were able to initiate nests could have been an artifact of taking nests measurements at different time points. For example, nests initiated by untreated queens were the first to exhibit brood emergence, which marked the end of the solitary phase and the experiment, and, thus, had less brood-rearing opportunity compared with treated queens. Despite having the shortest brood-rearing time, nests initiated by untreated queens were generally heavier, and significantly heavier than nests initiated by queens treated with 5 and 10 ppb syrup. Heavier nest weights may have been because of greater wax contributions and/or larger brood size; however, these factors were not measured. This method did not account for the differences in pollen consumption by larvae or for how much wax was produced for nest construction, but it provided an estimate of weight for comparison among treatments. Colony-level measures did not provide strong evidence for adverse effects on brood production despite treating pollen provisions (0.3, 1.7, 3.3, and 8.3 ppb), indicating that the main effects of imidacloprid treatment, in this study, were on queen survival and nest initiation rather than brood development. Effects on nest initiation may cause severe setbacks for bumble bee queens and colony development. Bumble bee colonies are annual and depend on rapid colony development to obtain the worker force necessary to maximize the production of future queens (gynes) and males for colony reproductive success (Lopez-Vaamonde et al. 2009). Seasonal constraints may, thus, limit the production of these critical reproductive individuals, suppressing population growth the following summer. Delayed colony development could also disrupt phenological synchrony with flowering plants, which are already affected by global climate change, and lead to further reduced plant–pollinator interactions affecting seed production and ecosystem services (Rafferty et al. 2015, Forrest 2015).

The impact of neonicotinoids on bumble bee queen egg-laying behavior may be the underlying mechanism for reduced colony development in treated colonies (Fauser et al. 2017). These findings elucidate the importance of evaluating risk from neonicotinoids in different castes such as reproductive queens and males and nonreproductive workers, as they may exhibit differences in sensitivity to pesticides and differences at various life phases (Rortais et al. 2005, Mommaerts et al. 2010). Further research also is needed to

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examine whether bumble bee queens can fully recover and sustain brood production sufficiently to produce new reproductive queens and males at the end of the growing season, and whether worker bumble bees exposed to neonicotinoids during larval development can function properly as adults. This study highlights gaps in our knowledge about potential nontarget effects of neonicotinoids and the need for research addressing the complex life histories and natural behaviors of different bees in regards to pesticide risk assessment and regulatory decisions. In addition, our results illustrate a need for protecting pollinators from neonicotinoids, particularly in the early spring when bumble bee queens and other wild bees are vulnerable to stressors during solitary phases of nest initiation and foraging.

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