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Sublethal imidacloprid effects on honey bee flower choices when foraging

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Abstract Neonicotinoids, systemic neuro-active pesticides similar to nicotine, are widely used in agriculture and are being investigated for a role in honey bee colony losses. We examined one neonicotinoid pesticide, imidacloprid, for its effects on the foraging behavior of free-flying honey bees (*Apis mellifera anatoliaca*) visiting artificial blue and white flowers. Imidacloprid doses, ranging from 1/5 to 1/50 of the reported LD_{50}, were fed to bees orally. The study consisted of three experimental parts performed sequentially without interruption. In Part 1, both flower colors contained a 4 µL 1 M sucrose solution reward. Part 2 offered bees 4 µL of 1.5 M sucrose solution in blue flowers and a 4 µL 0.5 M sucrose solution reward in white flowers. In Part 3 we reversed the sugar solution rewards, while keeping the flower color consistent. Each experiment began 30 min after administration of the pesticide. We recorded the percentage of experimental bees that returned to forage after treatment. We also recorded the visitation rate, number of flowers visited, and floral reward choices of the bees that foraged after treatment. The forager return rate declined linearly with increasing imidacloprid dose. The number of foraging trips by returning bees was also affected adversely. However, flower fidelity was not affected by imidacloprid dose. Foragers visited both blue and white flowers extensively in Part 1, and showed greater fidelity for the flower color offering the higher sugar solution reward in Parts 2 and 3. Although larger samples sizes are needed, our study suggests that imidacloprid may not affect the ability to select the higher nectar reward when rewards were reversed. We observed acute, mild effects on foraging by honey bees, so mild that storage of imidacloprid tainted-honey is very plausible and likely to be found in honey bee colonies.

Keywords *Apis mellifera* · Foraging behavior · Neonicotinoids

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Introduction

Animals, forming a diverse global pollination network, pollinate about 90 % of modern angiosperms. Of great concern to agronomists, and civilization as a whole, is that the stability of this worldwide network may decline with contemporary alterations of pollinator communities (Kearns et al. 1998). In fact, the total impact of pollination services on ecosystems and agriculture remains unknown because its effects are far-reaching and replacement of the services are difficult to assess (e.g. Allsopp et al. 2008; de Lange et al. 2013). Nevertheless, it is estimated that pollination services are worth $5–40 billion in the USA alone, and if ecosystem services are included in the assessment, up to $200 billion (Kearns et al. 1998; Calderone 2012). With about 25,000 species globally, the Apiodea, (bees) are key agents of ecosystem and agricultural pollination services in diverse habitats (Michener 2007). A shortage of bee pollinators from increased agricultural demand is exacerbated by a decline in wild bee pollinators and

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managed honey bee colonies. To meet the rising demand of food and to account for the decrease in crop yield from declining pollination services, it is estimated that increased land use will only offset about 3–8 % of the increasing food demand, especially in developing countries (Aizen et al. 2009; Aizen and Harder 2009).

Leading hypotheses for colony collapse disorder (CCD) link sublethal exposure to pesticides and other environmental factors, such as parasitic infections and habitat loss, to honey bee losses and pollinator declines in general (Brittain et al. 2010; Creswell et al. 2012a; Bryden et al. 2013; Cressey 2013). The sub-lethal stress (SLS) model predicts that sublethal stress affects the behavior of individual bees of a colony, which reduces bee reproductive rates and colony strength and persistence (Bryden et al. 2013). The SLS Model also predicts variation in colony persistence, whether a colony survives or goes extinct because of stress, depends on initial conditions and can explain contrasting results observed in previous studies (Desneux et al. 2007; Gill et al. 2012; Goulson 2013; Lu et al. 2014). Pesticide studies sometimes yield inconsistent results because studies have used different methods (see Cresswell 2011; Carreck and Ratnieks 2014 for reviews). Such inconsistencies, combined with difficulties in understanding responses to multiple stressors, could be why the discovery of the underlying causes of CCD has remained so elusive.

Neonicotinoid pesticides are currently the most widely used insecticides in the world and their increase in use correlates with declines in bee pollinators (review: Goulson 2013). Although concentrations of neonicotinoids in bee food sources are typically low (e.g. Rortais et al. 2005; Stoner and Eitzer 2012), the combination of chronic exposure to sublethal doses from diverse sources and other stressors fits the criteria for CCD as predicted by the SLS model. The importance of pollinators for food production is most critical in countries that use industrialized agriculture (Brittain et al. 2010; Creswell et al. 2012b). In the EU and UK, the need for pollinator services for food production has grown about five times faster than the pollinator stocks (Breeze et al. 2011, 2014). Other countries are facing similar pollinator shortages for food production (Potts et al. 2006; Dag 2009). Once established, pollinator shortages may be difficult to overcome because species-specific susceptibility to neonicotinoid pesticides and our relatively poor knowledge about the effect of neonicotinoids on bee pollinators in natural situations undermine effective pollinator management. While variation in life history traits, genetics, and mode of pesticide exposure may cause species of bees to respond differently to neonicotinoids, honey bees are particularly sensitive to pesticides (e.g. Devillers et al. 2003; Arena and Sgolastra 2014). The sensitivity of honey bees to pesticides, combined with their generalist foraging strategy and breadth of their activity season, offer unique opportunities to study the effect of neonicotinoids on pollinators and improve pollinator management.

Imidacloprid is applied to a wide range of crops, resulting in diverse sources of contaminated nectar and pollen upon which honey bees may forage (e.g. Rortais et al. 2005; Stoner and Eitzer 2012). One particularly alarming mode of exposure is through corn seedling guttation, where imidacloprid exposure can easily exceed the LD_{50} for honey bees (Girolami et al. 2009). Imidacloprid persists in plant tissues 30–100 days after application (DeGrandi-Hofman et al. 2012; Lu et al. 2012). Even though neonicotinoid concentrations found in nectar and pollen are in minute quantities compared to the honey bee LD_{50} value, growing evidence suggests that these low doses have negative effects on honey bee colonies (Aliouane et al. 2009; Gill et al. 2012; reviewed in Cresswell 2011; Cressey 2013). Recent evidence suggests that sublethal doses of neonicotinoids also affect the overwintering survival of colonies (Lu et al. 2012, 2014). This is consistent with the SLS Model for social bees where imidacloprid reduces fecundity at doses much lower than the LD_{50} value (Bryden et al. 2013). For example, bumblebees exposed to field-realistic doses of imidacloprid, well below the LD_{50} value, demonstrated a significant reduction in growth rate and produced 85 % fewer queens (Whitehorn et al. 2012). These studies underscore the importance of studying the effects of both acute and chronic sublethal doses of imidacloprid in honey bees (review: Desneux et al. 2007). Moreover, diverse experimental conditions and methodologies with different endpoints will contribute to a strong inference approach that account for competing hypotheses (sensu Wenner 1989).

The use of neonicotinoids has been prohibited in some EU countries, such as France, until December 2014 (Cressey 2013). The final decision for the use of these particular pesticides will be based on the accumulation of evidence from the latest research on neonicotinoid insecticides. Therefore, the aim of our study was to investigate the effects of sublethal imidacloprid doses on foraging behavior of the Anatolian honey bee (*Apis mellifera anatoliaca*), which is the most important subspecies of honey bee adapted to extreme climates in Anatolia (Ruttner 1988; Kandemir et al. 2000).

Materials and methods

We used free-flying honeybees, *A. mellifera anatoliaca*, foraging outdoors on artificial flower patches from an apiary with 40 Langstroth hives and 50 mini hives, totaling 90 colonies. Colonies were treated with Amitraz strips for *Varroa* mites in the fall of 2012. All colonies were healthy,

and contained a queen before and during the experiments. An artificial flower patch consisted of 36 (18 blue and 18 white) flowers, randomly placed 75 mm apart in a 6 × 6 Cartesian coordinate system in rows and columns, on a brown pegboard. An artificial flower was a 28 mm × 28 mm Plexiglas square, 6 mm thick, on a 90 mm long 5 mm diameter dowel. A 5 mm diameter, 5 mm deep well was in the center of each flower, and was manually loaded with nectar reward. Flowers of different colors were created by painting the lower surface of the flowers with blue or white enamel paint (Testors™ paint Nos. 1208 blue, 1245 white). The reflectance spectra for the paints, and a color hexagon depicting how these colors are perceived by the honeybee, can be found in Hill et al. (1997). Flowers were washed with unscented detergent, triple rinsed using deionized water, and allowed to dry between trials. Between parts of an experiment, a new set of flowers was used (e.g. Wells and Wells 1986; Çakmak et al. 2009; Abramson et al. 2013). These methods do not guarantee that flower odors have been eliminated completely from each artificial flower after being washed, because honey bees can perceive odors at concentrations <1 ppb. Creating a completely odor free foraging board for honey bees in an open environment is impractical (review: Abramson et al. 2012), and not needed. However, the methods used do insure there was not an odor bias between blue and white flowers, which is critical aspect to take account of in any choice experiment (Sanderson et al. 2013).

Foragers from ten-frame hives in the apiary were trained in trials conducted from 23 July to 10 August 2013 to fly 50 m to the experiment location where there was a clear petri dish containing clove-scented 1 M sucrose solution (5 µL/L clove oil). The petri dish was removed and replaced with an artificial flower patch where each blue and white flower contained 10 µL of unscented 1 M sucrose solution as a reward (e.g. see: von Frisch 1967; Wells and Wells 1986; Seeley 1995; Menzel 2001; Srinivasan 2010; Avarquès-Weber and Giurfa 2013; Amaya-Márquez et al. 2014). The bees used in our experiment were uniquely marked on the thorax with enamel paint (Seeley 1995) and randomly assigned to a treatment while additional bees were removed from the experimental system. Unmarked recruit bees were captured and kept in cages with bee candy until the end of the experiments.

A trial had three phases performed sequentially without breaks: (1) crop attachment, (2) pesticide, and (3) test phase (Fig. 1). The *Crop Attachment Phase* lasted 30 min and offered bees 10 µL of unscented 1 M sucrose in each flower. Flowers were refilled with the same reward consumed after visitation by a bee. In the *Pesticide Phase*, bees were captured on its first flower, before consuming the reward, immediately fed 5 µL of unscented 1 M sucrose solution containing a randomly assigned treatment solution (an imidacloprid dose or control). The 5 µL droplet was hand fed using a micro-pipetter, which allowed us to ensure that the entire amount was consumed. Bees readily ingested the imidacloprid-sugar solution. Bees were held in captivity for 15 min, and then released. The flower patch remained in place, without nectar rewards, for an additional 15 min. The 30-min. pesticide phase allowed the pesticide to be ingested by bees. The *Test Phase* consisted of 3 parts given sequentially without breaks between treatments. Part 1 (30 min) offered bees 4 µL of unscented 1 M sucrose solution in both flower colors. Part 2 (45 min) offered bees 4 µL of unscented 1.5 M sucrose solution in each blue flower and 4 µL of unscented 0.5 M sucrose in each white flower. In Part 3 (45 min) the rewards associated with flower color were reversed from Part 2, so that white flowers offered the 1.5 M sucrose solution reward. The switch in reward allowed for comparison of reward reversal learning. The food reward in the flowers was refilled every time a bee consumed it after a foraging visit (Fig. 1).

Treatments of imidacloprid doses were based on the LD_{50} value calculated from mortality from acute contact exposure from a topical application (Iwasa et al. 2004). Five treatments were used: 7.20 ng/bee imidacloprid (40 % of the LD_{50}; N = 11), 1.80 ng/bee imidacloprid (10 % of the LD_{50}: N = 38), 0.72 ng/bee imidacloprid (4 % of the LD_{50}: N = 22), 0.36 ng/bee imidacloprid (2 % of the LD_{50}; N = 23), and 0.00 ng/bee imidacloprid (no pesticide: the negative control; N = 93). The large number of negative control bees resulted from conducting a paired experimental design. Each trial paired one bee from the negative control (no pesticide) with one bee from the treatment. Multiple trials of each treatment were performed, each with a new set of bees, in random order with respect to imidacloprid treatment. Each trial was started with approximately 6–8 bees but only 4 or fewer bees completed the 3 parts of the test phase. A total of 188 bees were used in 47 trials. Bees receiving 7.20 ng/bee imidacloprid served as a positive control because, at this dose harnessed bees, in the laboratory previously demonstrated a significant effect after ingestion (Hranitz Unpublished). For the bees that returned to foraging on the artificial flower patches, flower color sequence that each bee visited and the number of trips a bee made from the hive was recorded by an observer.

A single Chi square test (2 columns, return vs not-return, and 5 rows, treatment) was used to compare the number of bees that returned to forage imidacloprid treatment. Non-returning bees never returned to the flower patch after being released from pesticide treatment. We tested the dose–response relationship for the return of bees after the pesticide dose was administered by linear regression (significance test of the regression: ANOVA).

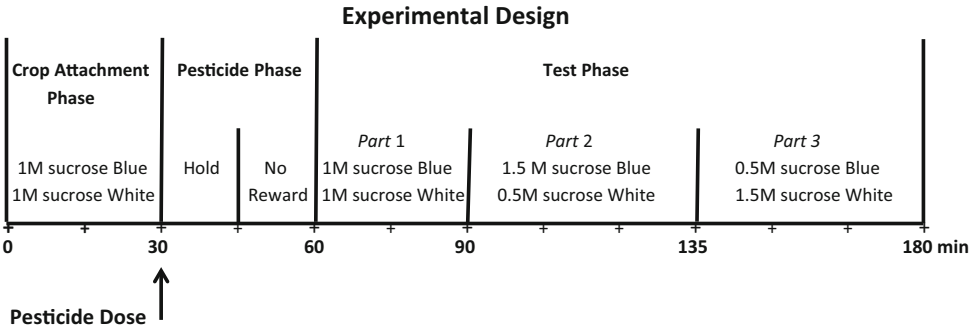

Fig. 1 A schematic of the experimental design utilized. Each experiment consisted of three phases, which were given sequentially without a break. The crop attachment phase conditioned bees to visit the artificial flower patch. In the pesticide phase we administered imidacloprid and allowed it to be absorbed by the bee. The test phase followed, which examined forager flower-color fidelity under different reward scenarios

A repeated-measures MANOVA was used to test for flower-color fidelity changes in response to different rewards associated with flower color in the Phase 3 of experiment. To normalize the data, an arcsine square-root transformation was used on the proportion of blue flowers visited for each of the three test-phase parts. This transformation normalizes the relative frequency data (Sokal and Rolf 1995). Dose of imidacloprid (1.80, 0.72, 0.36 or 0 ng), experimental part (1, 2, and 3), and interaction effects were tested (Sall and Lehman 1996). None of the 40 % LD_{50} (7.2 ng) bees returned to the artificial flower patches, so this treatment was removed from analysis. We used an ANOVA to test the effect of imidacloprid dose (1.80, 0.72, 0.36 or 0 ng) on number of flowers visited and number of trips bees made (Test Phase, Parts 2 and 3).

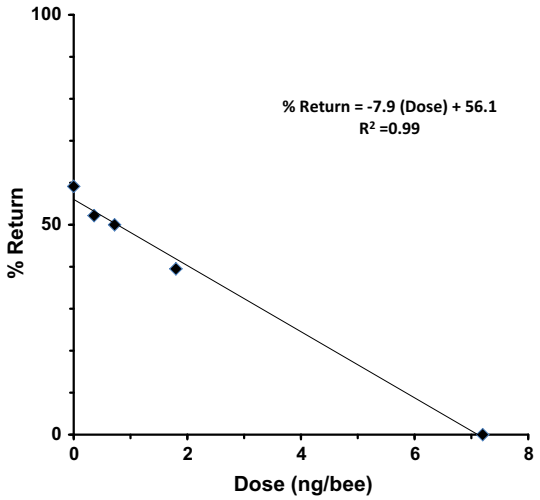

Fig. 2 Percentage of foragers returning in the test phase plotted as a function of imidacloprid dose (7.20 ng imidacloprid = 40 % LD_{50}; 1.80 ng = 10 % LD_{50}; 0.72 ng = 4 % LD_{50}; 0.36 ng = 2 % LD_{50}; 0 ng = 0 % LD_{50}). Regression line depicted ($r^2 = 0.992$; N = 188 bees)

Results

Of the 188 bees used in the experiment, 113 bees (60.1 %) returned to the artificial flower patches and visited flowers. Dose had a significant effect on number of bees that resumed foraging behavior after being fed and held in captivity (χ^2= 15.93, df = 4, $P < 0.01$; Power = 0.9999). The percentage of bees that successfully returned to foraging on the artificial flower patches decreased with increased imidacloprid dose (F = 391.6; df = 1.3; $P < 0.0003$; with $R^2 = 0.992$; Power = 0.9999). None of the bees treated with a dose of 7.20 ng (40 % LD_{50}) successfully returned to the artificial flower patch after being released (Fig. 2).

In order to eliminate bias, only bees that completed all three Test Phase parts were used in the analysis of trip rate, flower visitation rate and ability to choose the greater reward. Of the 113 bees returning, 94 (83.2 %) completed all three Test Phase parts. The number of flowers visited by returning bees that foraged in Test Phase Parts 2 and 3 did not differ significantly between treatments (F = 2.2953; df = 3,69; P = 0.0856; Power = 0.5548). The number of foraging trips from the hive to the artificial flower patch differed between all imidacloprid and control treatments for bees in Parts 2 and 3 of the Test Phase (F = 4.496; df = 3,69; P = 0.0061; Power = 0.8634), but the numbers of foraging trips were similar among bees in imidacloprid treatments (F = 0.5450; df = 2,69; P = 0.5856; Power = 0.1313). On average, bees that were fed any dose of imidacloprid made about 15 % fewer trips (Figs. 3, 4).

When examining low dose effects of imidacloprid on foraging decisions, we found that there was a Test Phase effect (F = 137.23; df = 2,68; P = 0.0001; Power = 0.9999), but dose (F = 0.313; df = 3,69; P = 0.8158; Power = 0.1487) and interaction (F = 1.888; df = 3,69;

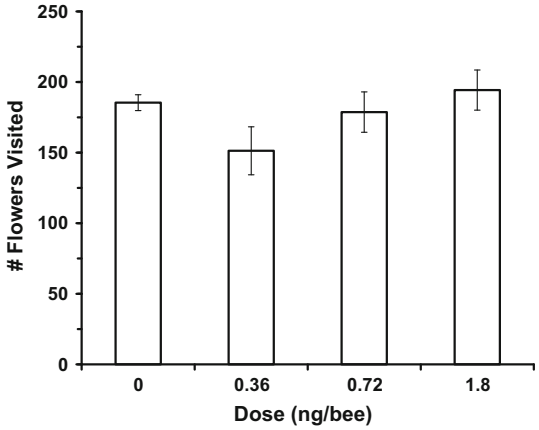

Fig. 3 Total number of flowers visited per bee for each dosage group (mean with SE *bars*) in parts 2 and 3 of the experiment. Only bees that returned to foraging after the pesticide phase and completed all three parts of the test-phase were included in the final analysis (N = 94 bees). There was no significant difference between dose treatments

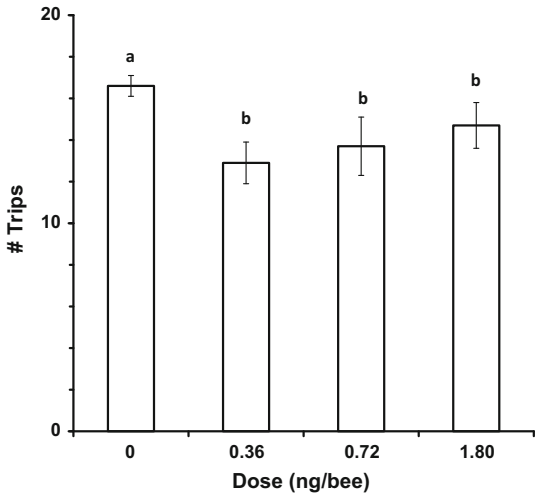

Fig. 4 Total number of trips made to the artificial flower patch per bee for each dosage group (mean with SE bars) in parts 2 and 3 of the experiment. Only bees that returned to foraging after the pesticide phase and completed all three parts of the test-phase were included (N = 94 bees) in the final analysis. Significant differences at the alpha = 0.05 level are denoted with letters

P = 0.0872; Power = 0.4210) effects were not significant. In Part 1, when both flower colors offered 1 M sucrose solution rewards, treated bees visited blue flowers less than control (mean 468 for control vs. 263 treated bees). In Part 2, bees showed high fidelity to blue flowers when blue offered the 1.5 M sucrose reward and switched fidelity when white offered the 1.5 M sucrose reward (Fig. 5). Bees treated with imidacloprid distinguished between high and low molarity nectar rewards, but larger sample sizes are needed to increase the statistical power of the hypothesis that imidacloprid has "no effect" on foraging decisions in honeybees.

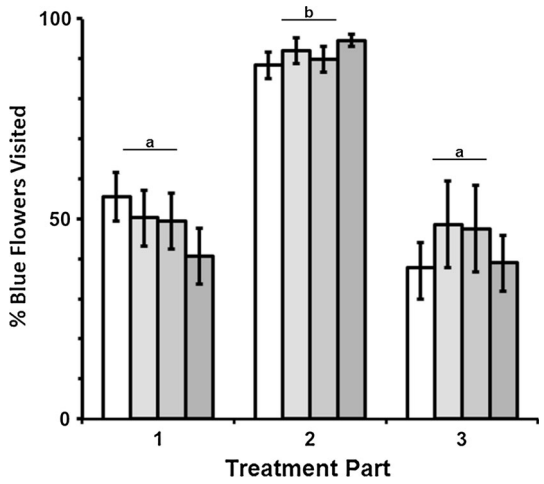

Fig. 5 Flower color fidelity of foragers under different reward scenarios (N = 94 bees). Bars from left to right in each part of the experimental Part represent the imidacloprid doses [0 ng (0 % LD_{50}), 0.36 ng (2 % LD_{50}), 0.72 ng (4 % LD_{50}) and 1.80 ng (10 % LD_{50})]. Depicted is the mean (with SE *bars*) percentage of blue flowers visited. Only bees that returned to forage after the pesticide phase were included. Part 1 offered bees 1 M sucrose solution in both flower colors. Part 2 offered bees 1.5 M sucrose in blue and 0.5 M sucrose solution in white flowers and part 3 offered bees 0.5 M sucrose in blue and 1.5 M sucrose solution in white flowers. Significant differences at the alpha = 0.05 level are denoted by *letters*

Discussion

Our study examined the effects of field-realistic sublethal doses of the neonicotinoid pesticide imidacloprid using controlled conditions in artificial flower patches in more natural conditions than laboratory studies. Even the highest dose we used (7.20 ng/bee) is within the range of exposures found in agricultural settings and prior studies of the effects of imidacloprid on foragers (Colin et al. 2004; Rortais et al. 2005; Bonmatin et al. 2005; Feltham et al. 2014; Stoner and Eitzer 2012; Blacquiere et al. 2012; Carreck and Ratnieks 2014; Laycock et al. 2014; Fischer et al. 2014). Imidacloprid adversely affected two components of foraging in our study: the number of returning bees and the number of foraging trips. The number of returning bees decreased linearly with increasing imidacloprid dose ingested, with no bees continuing to forage at a dose that is only 40 % of the LD_{50} (Fig. 2). Roughly 8 % fewer bees returned for each additional 1 ng imidacloprid ingested. Bees that continued to forage after ingesting imidacloprid made about 20 % fewer foraging trips from the hive, but this effect was not dose dependent. However, bees that ingested imidacloprid visited a few more flowers per trip than the control bees, which compensated for the fewer foraging trips when considering the average number of flowers visited. Whether or not imidacloprid dose affected the total number of flowers visited remains to be rigorously

tested because the statistical power (0.5548) of this analysis was low. Larger sample sizes are needed to test for no difference between the 2 % of the LD_{50} value and other treatments (including negative control). Bees altered their fidelity flower color as expected, preferring the flower color that yielded the highest sucrose solution reward. They thus showed high fidelity to blue flowers when it offered a 1.5 M sucrose solution reward, and abandoned it for white flowers when they offered the greater molarity reward. These results suggest that imidacloprid does not disrupt the associative learning and memory required to pair a flower color with a sucrose solution reward and discriminate between two different reward sizes but, power analysis revealed low confidence in accepting the null hypothesis and the need for larger sample sizes. In a related study, the sublethal dose of (48 ppb, 4 replicates per treatment and 40 honey bees) imidacloprid reduced visual learning ability of the honey bees and only 40 % of the imidacloprid treated bees made the right decision to reach to the reward compared to the control in T-tube maze assay with yellow and blue colors (Han et al. 2010).

In Test Phase Part 1, it is interesting to note that when both flower colors offered the same reward as 1 M sucrose solution, bees given 20 and 10 % of the LD_{50} value of imidacloprid visited blue flowers less often than white flowers (Fig. 5). In Test Phase Part 2, the differences in flower color fidelity were small where blue flowers offered the higher caloric reward. There was no significant difference among the dose treatments in each part of the test phase, but there was among the three parts, as indicated in Fig. 5, which suggests bees showed preference for the flowers with higher reward irrespective of their color. However, even if bees receiving imidacloprid actually do show higher fidelity to blue flowers here, it is less than a 5 % fidelity difference which would translate to a minimal energetic gain for the colony. Similarly, flower color fidelity differences were not large in Part 3 of the Test Phase, and we did not detect a significant difference among dose treatments. Unlike Part 2 of the Test Phase, the bees receiving lower doses of imidacloprid have a relatively higher preference for the blue flowers even though this no longer offers the higher reward. This lag in adjusting could be due to the effects of pesticide as it acts to disrupt acetylcholine receptors in the brain. A large sample size is needed to determine if very small imidacloprid doses impede the learning required to associate the new color which is paired with the large reward. Regardless, the effects of the pesticide do not impede foraging enough to prevent tainted nectar from being brought back to the hive, which would result in the buildup of substantial honey stores with imidacloprid in a field situation.

This same scenario is predicted by the elegant mathematical model of Rondeau et al. (2013), which estimates time-to-lethality due to prolonged imidacloprid ingestion. The model revolves around the fact that imidacloprid has a very high affinity to nicotinic-acetylcholine receptors in the insect's CNS, therefore the toxic effects can accumulate with chronic exposure even though most of the pesticide ingested is metabolized rapidly (5 h half-life). The model predicts that overwintering honey bee would be substantially affected by sub-lethal ingestion of imidacloprid found in stored honey due to their reliance on feeding on only stored honey to provide energy to survive the winter (Rondeau et al. 2013), and in fact this has been empirically demonstrated in field experiments (Sandrock et al. 2014; Dively et al. 2015).

Previous studies differ in the administration of imidacloprid and there are advantages and disadvantages in using each method. Some studies exposed bees to the pesticide using artificial flowers while other bees were fed individually and then monitored in the hive. A bee freely foraging on sucrose solution at an artificial feeder is a more realistic natural scenario than ours, resulting in pesticide being brought back to the hive with field realistic doses. However, feeder studies suffer from a lack of control in administering a known amount of pesticide to the bee because feeder studies do not record how much of the imidacloprid was absorbed by the bee, versus the amount shared by trophallaxis with hive mates or stored in the hive. Previous studies of individual feeding regimes, which delivered a controlled pesticide dose to bees, do not examine free-flying bee foraging decisions. While direct comparisons of results from this study with previous pesticide investigations are tentative, different experimental designs, taken together, reveal consistencies in the effects of imidacloprid on foraging, and shed light on the underlying mechanisms that might disrupt foraging.

Studies of different honey bee subspecies (*A. m. carnica*, *A. m. anatoliaca*, *A. m. ligustica*) reveal that imidacloprid doses of about 20–40 % of the LD_{50} for imidacloprid adversely affected the flight of foragers. This is manifested by lowered attendance of bees at feeders, fewer numbers of returning bees, reduced activity levels of bees in hives, and slower rate of flower patch visitation (Colin et al. 2004; Scholer and Krischik 2014), as seen in this study.

Methods that track individual bees (such as radio frequency tagging and radar tracking) reveal that components of navigation and cognitive processing during vector flight and homing flight can be disrupted in foragers intoxicated by sub-lethal doses of imidacloprid (Feltham et al. 2014; Fischer et al. 2014). However, foraging behavior over a prolonged time on pesticide-tainted nectar has been shown to be inconsistent and unpredictable when comparing to short-term studies. High doses of fipronil, for example, reduce the number of foraging trips continuously from the first day of exposure, but low doses do not. Interestingly,

after 4 days the low fipronil dose treatment, bees actually made more foraging trips per day (Decourtye et al. 2011). If this same trend holds true for imidacloprid, then the effects of chronic, low doses of the pesticide would be even more severe than what our short-term study predicts.

Imidacloprid intoxication at doses slightly higher (12.0–9.6 ng) than the highest dose used in our studies (7.2 ng) negatively impacts learning and memory association of scent with reward for both freely foraging bees and harnessed bees in PER experiments (Decourye et al. 2003; Ramirez-Romeo et al. 2005, 2008). Honey bees fed imidacloprid were slower learners when participating in a proboscis extension response (PER) conditioning test, but reached the same learning proficiency and subsequently abandoned the conditioned stimulus more rapidly when a reward was no longer paired with it (Ramirez-Romero et al. 2008). Thus, the PER results suggest that forager behavior would not substantially prevent the collection and foraging of tainted nectar by the colony as a whole. Our study specifically tested the ability of foragers to associate color with reward and imidacloprid did not display an adverse effect on the ability of bees to learn the association between reward and artificial flower color (Fig. 5). Our results suggest that the lower doses of imidacloprid (0.35–1.80 ng) do not interfere with the cognitive processes involved in foraging efforts.

Our results suggest the worst possible scenario for honey bee populations ingesting nectar with very low levels of imidacloprid. Foragers will continue to visit imidacloprid-treated crops, making regular trips to and from the hive, but only at a slightly slower rate. Cognitive processes of foragers would not be significantly compromised to prevent the continuation of foraging at these crops and the nectar's odor brought back to the colony will recruit more naïve bees (e.g. Johnson and Wenner 1966; Wenner et al. 1969; Wenner and Wells 1990) to visit the imidacloprid-tainted crop, thus increasing the neonicotinoid pesticide stores in honey. Afterward, when colonies need to overwinter and must rely on their honey stores, widespread poisoning of the colony by accumulated pesticides in the honey may occur. This would is cause widespread mortality (Rondeau et al. 2013; Lu et al. 2014). Ironically, if imidacloprid was very toxic to bees, and immediately killed foraging bees, the colony as a whole would fare better because recruitment of more foragers would not occur. With a high-toxicity scenario, colony losses might actually be limited to a few foragers, and the colony would remain free from pesticides.

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Compliance with ethical standards

Conflict of Interest There is no conflict of interest.

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