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Source: Environmental Entomology, 44(6):1544-1552.
Published By: Entomological Society of America
URL: http://www.bioone.org/doi/full/10.1093/ee/nvv127

Sublethal Effect of Imidacloprid on *Solenopsis invicta* (Hymenoptera: Formicidae) Feeding, Digging, and Foraging Behavior

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Environ. Entomol. 44(6): 1544–1552 (2015); DOI: 10.1093/ee/nvv127

ABSTRACT There is increasing evidence that exposure to neonicotinoid insecticides at sublethal levels impairs colonies of honeybees and other pollinators. Recently, it was found that sublethal contamination with neonicotinoids also affect growth and behavior of ants. In this study, we exposed red imported fire ants, *Solenopsis invicta* Buren, to sublethal dosages of dietary imidacloprid and investigated its effect on ant feeding, digging, and foraging behavior. *S. invicta* consumed significantly more sugar water containing 0.01 µg/ml imidacloprid than untreated sugar water. Ants fed with 0.01 µg/ml imidacloprid also showed significantly increased digging activity than ants fed with untreated sugar water. However, imidacloprid at $\geq$ 0.25 µg/ml significantly suppressed sugar water consumption, digging, and foraging behavior. These results indicate that imidacloprid at sublethal concentrations may have a significant and complicated effect on *S. invicta*.

KEY WORDS *Solenopsis invicta*, neonicotinoid, sublethal concentration, feeding, recruitment

Neonicotinoids are widely used insecticides (Jeschke et al. 2011). Imidacloprid, a common neonicotinoid insecticide, was routinely used on rice, sunflower, maize, potatoes, vegetable, sugar beets, fruit, cotton, hops, and turfs as seed dressing, soil treatment, and foliar spray (Elbert et al. 2008). Residues and degradation of imidacloprid in soils, vegetable, food, and water have been well investigated. The half-life of imidacloprid can range from 28.7 to 47.8 d in three different soil types—Gangetic alluvial soil, lateritic soil, and coastal alkaline soil (Sarkar et al. 2001). In vineyard, 86 d after the application at recommended and double the recommended dosages (Confidor 200SL at 400 and 800 g ha^{-1} 1,000 $liter^{-1}$ of water, 0.008 and 0.016% a.i.), the residue level of imidacloprid in soil reached 0.12 and 0.22 mg/kg, respectively (Arora et al. 2009). When the field was treated with imidacloprid (seed dressing), imidacloprid was detected at the end of the cultivation in the soils with various compositions (mean = 12 µg/kg; Bonmatin et al. 2003). In a study on the residues of imidacloprid in vegetables, fruits, and water samples collected from the West Bank, Palestine, the highest and lowest imidacloprid concentrations were found in eggplant (0.46 mg/kg) and green beans (0.08 mg/kg), respectively (Daraghmeh et al. 2007). Neonicotinoids were also detected in bee pollen (Mullin et al. 2010, Blacquière et al. 2012).

Worldwide population declines of many pollinators may be caused by the extensive use of neonicotinoids (Decourtye and Devillers 2010). Neonicotinoids at sublethal levels can effect learning and memory of honeybees (Decourtye et al. 2004, Aliouane et al. 2009), and decrease their foraging success (Blacquière et al. 2012, Henry et al. 2012). After feeding on dietary imidacloprid at 6 to 12 ppb for 2 wk, the growth rate and queen production of colonies of the bumble bee, *Bombus terrestris* (L.), were significantly reduced (Whitehorn et al. 2012). On exposure to field-realistic doses of imidacloprid, pollen foraging efficiency, wax pot production, and nectar storage of bumblebees, *Bombus* spp., were reduced (Felttham et al. 2014, Scholer and Krischik 2014), and foraging preferences for the flower types were altered (Gill and Raine 2014).

Among the few studies on ants, it was found that exposure to sublethal neonictinoids can change ant behavior and colony fitness. For instance, interspecific aggressive behavior and colony fitness of Southern ants, *Monomorium antarcticum* (Smith), and Argentine ants, *Linepithema humile* (Mayr), were altered after feeding on aqueous honey solution containing 1.00 µg/ml imidacloprid (Barbieri et al. 2013). Imidacloprid at 10 ng/ant reduced self-grooming behavior of *Acromyrmex subterraneus subterraneus* (Forel) (Galvanho et al. 2013). In this study, we investigated the effect of dietary imidacloprid at concentrations ranging from 0.01 to 1.0 µg/ml on feeding, digging, and foraging behavior of red imported fire ants, *Solenopsis invicta* Buren, as these behaviors are essential to colony development. Due to its significance in public health, agriculture, and biodiversity, *S. invicta* is considered as one of the worst invasive species

We declare that no conflict of interests exists. Mention of trade names or commercial products in this publication is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U. S. Department of Agriculture.

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in the world (Lowe et al. 2000). *S. invicta* has become one of the most well studied ant species in the world (http://www.myrmecos.net/2009/01/25/the-most-studied-ant-species-are-either-trampy-or-european/, last accessed 4 August 2015), making it an ideal model to evaluate the effect of neonicotinoid insecticides on ants.

Materials and Methods

Insects. Red imported fire ant *S. invcta* colonies were collected from Leroy Percy Wildlife Management Area, Washington County, MS. Pesticide use is not allowed in this area. Species identity was confirmed using ant cuticular hydrocarbon characteristics (Ross et al. 1987). Colonies were fed with 10% w/w sugar water and frozen crickets. All colonies were reared under laboratory condition (28°C and 45% relative humidity [RH]) for at least 1 wk prior to experiment. Polygyne colonies were used in this study.

Insecticides and Sublethal Dosage. Imidacloprid (>99.9% purity, Sigma-Aldrich, St. Louis, MO) was directly dissolved in 5% w/w sugar water. Based on previous studies (Barbieri et al 2013, Galvanho et al 2013) and our preliminary experiments, five concentrations of imidacloprid—0.01, 0.05, 0.25, 0.50, and 1.00 μg/ml—were selected for feeding and digging bioassays and two concentrations—0.01 and 0.25 μg/ml—for foraging bioassays. Solutions were freshly prepared before each experiment.

Feeding Bioassay. The effect of imidacloprid on sugar water consumption was evaluated in both nonchoice and choice bioassays. In nonchoice feeding bioassay, a colony was divided into four subcolonies with same weight, and each subcolony had at least one queen. Ants in these subcolonies were first starved for 48 h before experiments. Each of subcolonies then received either control sugar water or a single concentration of imidacloprid in 5% (w/w) sugar water, which was provided in a 15-ml test tube that was plugged with a cotton ball. Ants were allowed to feed ad libitum for 7 d. There were two separated experiments. Imidacloprid at 0.25, 0.50, and 1.00 μg/ml was tested in experiment 1 and 0.01, 0.05, and 0.25 μg/ml in experiment 2. Because different colonies were used in these two experiments, one concentration, 0.25 μg/ml, was included in both experiments for the purpose of comparison. The feeding tubes were weighed before and after the experiment and average daily consumption (mg sugar water/gram ant/day) was calculated. The daily consumption was adjusted for the natural water evaporation from the sugar water. The amount of water evaporated was estimated by weighing three tubes that contained each dosage of imidacloprid before and after they had been kept at the same condition for 7 d. Every colony was used as a replicate, and four colonies were used for each experiment. The weight of every colony was more than 20 g, and 1 g *S. invicta* workers contained about 1,000 workers.

In choice feeding bioassay, a colony was divided into five subcolonies with same weight, and each subcolony contained at least one queen. Two feeding tubes were provided to each subcolony simultaneously. One tube contained 15 ml 5% sugar water and the other tube contained 15 ml sugar water with 0.01 μg/ml imidacloprid. Ants were starved for 48 h before the experiment. The feeding tubes were weighed before and after the 7-d feeding period and average daily consumption (mg sugar water/gram ant/day) was calculated for each tube. Five colonies were used.

Digging Bioassay. Digging is an intrinsic behavior of *S. invicta* workers. They will dig whenever a suitable substrate is available. The effect of imidacloprid on ant digging effort was evaluated using the difference in the amount of sand removed between ants fed on sugar water containing imidacloprid and those fed on untreated sugar water. Two bioassays were conducted: bioassay 1, in which sugar water supply in both control and imidacloprid treatments were unlimited before digging bioassays, and bioassay 2, in which sugar water supply in control but not in imidacloprid treatments were limited. In bioassay 1, fire ants were found to consume significantly less sugar water containing ≥0.25 μg/ml imidacloprid than control. Because sugar intake itself might affect ant digging performance, to evaluate the effect of imidacloprid, the possible effect of sugar intake must be minimized. To accomplish this, bioassay 2 was included.

For bioassay 1, subcolonies from the nonchoice feeding bioassays were used. Those subcolonies had fed on sugar water containing 0.00, 0.01, 0.05, 0.25, 0.50, or 1.00 μg/ml imidacloprid for 7 d. The digging bioassay apparatus developed by Chen and Allen (2006) was used for the digging study. The apparatus consists of one Petri dish (9.0 by 2.0 cm^2) and a capped Wheaton liquid scintillation vial (2.8 by 6.1 cm^2) right underneath it (Supp. Fig. 1 [online only]). There was a 3-mm access hole between the Petri dish and the vial. The inside wall of the Petri dish was coated with Fluon to prevent the ants from escaping. Sand (Premium Play Sand, Plassein International, Longview, TX) was washed using distilled water and dried at 150°C until its weight become constant. Moistened sand was prepared by placing 120 g of sand in a 1,000-ml beaker, adding 10.45 ml of distilled water into the beaker and stirring sand in the beaker with a glass rod to ensure the homogenous mixture of sand and water. Two separated experiments were conducted in bioassay 1. Imidacloprid at 0.25, 0.50, and 1.00 μg/ml was tested in experiment 1 and 0.01, 0.05, and 0.25 μg/ml in experiment 2. Ants fed on sugar water without imidacloprid were used in control. Thirty fire ants workers were transferred into the Petri dish of the digging bioassays apparatus. After 24 h, the sand on the Petri dish were collected, dried at 150°C until its weight became constant, and weighed. The total number of died workers in vials and in the Petri dish was recorded. Each treatment and control was replicated five times for each colony and four colonies were used. All tests were conducted in a controlled environment at 27.0 ± 0.3°C and 60.0% RH.

In bioassay 2, colonies were treated as described in nonchoice feeding bioassay except sugar water supply in control was limited, aiming to reduce the difference in sugar water intake between treated subcolonies and

untreated subcolonies. Two separated experiments were conducted: 0.25, 0.50, and 1.00 μg/ml imidacloprid in the experiment 1 and 0.01, 0.05, and 0.25 μg/ml imidacloprid in the experiment 2. For experiment 1, the daily supply of sugar water in control was 0.20 g/g ants for all 7 d. For experiment 2, the daily supply of sugar water in control was unlimited for the first 3 d, but limited to 0.10 g/g ant for the rest 4 d. The bioassay procedure was the same as that for bioassay 1.

Foraging Bioassay. Ant foraging activities before and after exposure to imidacloprid were measured. The bioassay consisted of two experiments: pre-exposure experiment and post-exposure experiment. For the pre-exposure experiment, colonies were first depleted of food supply for 2 d and then provided with 5% sugar water for 7 d. Sugar water was supplied by a feeding tube that was made from a 5-ml microfuge tube plugged with a cotton ball. Daily sugar water consumption was measured. The weight of colony was also recorded daily. After 7 d, ants were allowed to forage for 24 h. Sugar water was replaced by untreated water during this 24-h period. An artificial nest was connected to a foraging arena (round plastic container, diameter 25.0 cm, height 9.0 cm) with a 60.0-cm bridge (Supp. Fig. 2 [online only]). A square container (9.0 by 9.0 by 0.8 cm^3) with baits was placed in the foraging arena about 18.0 cm to the bridge. Baits consisted of zein-enhanced corn grit with 20.0% soybean oil (Crisco pure vegetable oil, the J.M. Smucker Company, Orrville, OH). Discovery time and recruitment time were recorded. The discovery time was the time period from when the bridge was set up between the tray and foraging arena to when the first ant contacted bait. The recruitment time was the time period from when the first worker contacted the bait to when 10 foragers showed up on the bait. After 10 foragers showed up on the bait, the number of ants on the bait container was estimated at 10, 20, 40, 60, 120, 150, and 180 min. Walking speed of workers was also measured by recording the time needed for an ant to walk cross the 20.0 cm horizontal segment of the bridge. A total of 25 workers were measured for each colony. After 24 h, the square container with baits was removed and weighed. Amount of retrieved baits was measured. Because baits absorbed water during experiment, amount of retrieved bait was adjusted by the amount of absorbed water. Amount of absorbed water was calculated based on the mass change of corn grit under identical experimental condition. After pre-exposure experiment, these fire ant colonies were provided with water, 10% sugar water, and frozen crickets for 5 d. They were then used in post-exposure experiment. Again, food was first removed from the colonies. Two days later, colony was provided with sugar water containing either 0.01 or 0.25 μg/ml imidacloprid for 7 d. Ants were then allowed to forage for 24 h. The food discovery time, recruitment time, the time series of number of ants on the baits, walking speed, and amount of retrieved baits were measured. Five colonies were used for each dosage of imidacloprid. Each of the test colonies had at least one functional queen. All experiments were conducted in a climate-controlled room (28.0°C, approx. 60.0% RH).

Data Analysis. In feeding and digging bioassays, differences among control and treatments in daily sugar water consumption, number of dead ants after 24 h digging, and amount of removed sand were compared. All data were first checked for normal distribution by Shapiro–Wilk test and for homogeneity of variances by Levene's test. If the data were normally distributed and had similar variances, then one-way analysis of variance (ANOVA) was used to compare means among measured variables. When ANOVA results were significant, multiple comparisons of means were performed with least-significant difference (LSD) analysis. If data were not normally distributed, nonparametric Kruskal–Wallis test for comparing the median was applied. If the results of the Kruskal–Wallis test showed significant differences at the 0.05 significance level, Mann–Whitney test was used for pairwise comparisons. In choice feeding bioassay, a paired *t*-test was used to compare sugar water consumption between control and treatment. For foraging bioassays, all data were also checked for normal distribution. Differences in food discovery time, ant recruitment time, ant walking speed, and amount of retrieved baits between pre- and post-imidacloprid exposure were compared. When the data were normally distributed, *t* test was used; otherwise Mann–Whitney U test was used. Number of ants on the bait was analyzed using generalized linear model (GLM). All statistical analyses were performed in SPSS version 17.0 (SPSS, 1998).

Results

Feeding Bioassay. In nonchoice feeding bioassay, the daily sugar water consumption was significantly different among treatments and control (Fig. 1). In experiment 1, ants consumed significantly more untreated sugar water than those containing ≥0.25 μg/ml imidacloprid (Fig. 1A, $F_{3,12} = 14.346$, $df = 15$, $P = 0.0001$). However, there was no significant difference in sugar water consumption among groups fed on 0.25, 0.50, and 1.00 μg/ml imidacloprid. In experiment 2, fire ant consumed significantly more sugar water containing 0.01 μg/ml imidacloprid than untreated sugar water (Fig. 1B, $F_{3,12} = 3.820$, $df = 15$, $P = 0.039$). Despite consuming less sugar water, workers exposed to higher dose ingested larger amount of imidacloprid (Fig. 1A, $H = 11.575$, $df = 3$, $P = 0.009$; Fig. 1B, $H = 14.328$, $df = 3$, $P = 0.002$), except that 0.50 μg/ml imidacloprid treatment was not significantly different to either 0.25 μg/ml imidacloprid treatment ($U = 5.000$, $df = 1$, $P = 0.386$) or 1.00 μg/ml imidacloprid treatment ($U = 2.000$, $df = 1$, $P = 0.083$). In two-choice feeding bioassay, the daily sugar water consumption was always numerically larger in control, but the different among treatments and control was not statistically significant for all colonies (Table 1).

Digging Bioassay. In Bioassay 1, ants had unlimited sugar water supply in both control and imidacloprid treatments before the digging bioassay. For experiment 1 (0.25, 0.50, or 1.00 μg/ml imidacloprid), amount of removed sand and ant mortality are summarized in Table 2. There was a significant difference

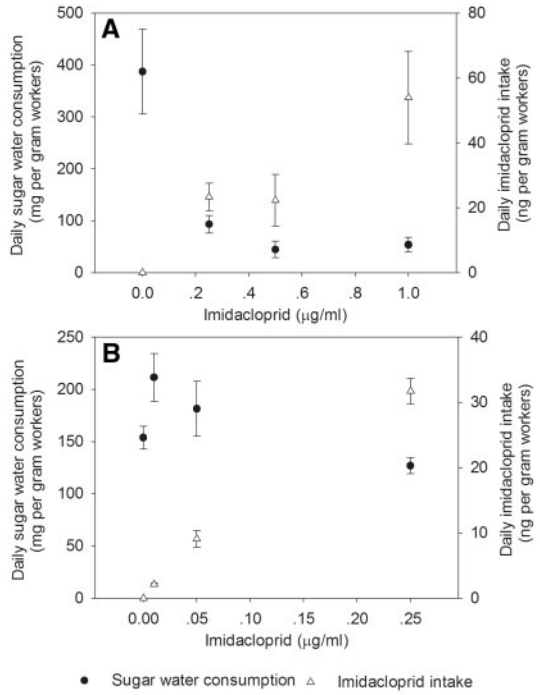

Fig. 1. Mean (± SE) daily sugar water consumption rate (black circular symbol) and mean daily imidacloprid intake (open triangle symbol) by *S. invicta* workers in experiment 1 (A) and experiment 2 (B) ($N = 4$).

Table 1. Consumption of sugar water and sugar water containing 0.01 μg/ml imidacloprid in a two choice feeding bioassay

Colony	Consumption (mg/g ants/d, mean ± SE)		*t*-value	*P*-value
	5% w/w sugar water	0.01 μg/ml imidacloprid		
Q	92.89 ± 15.53	63.30 ± 11.08	1.143	0.317
R	60.46 ± 10.40	54.04 ± 11.86	0.304	0.776
S	84.13 ± 11.41	56.46 ± 16.23	1.355	0.247
T	113.87 ± 44.34	58.72 ± 10.68	1.070	0.345

on the amount of removed sand among treatments and control for each colony (colony A: $H = 14.554$, $df = 3$, $P = 0.002$; colony B: $H = 9.937$, $df = 3$, $P = 0.019$; colony C: $H = 17.992$, $df = 3$, $P = 0.0001$; colony D: $H = 17.103$, $df = 3$, $P = 0.001$; colony E: $F_{3,16} = 14.663$, $df = 19$, $P = 0.0001$; colony F: $F_{3,16} = 6.076$, $df = 19$, $P = 0.006$; colony G: $H = 15.286$, $df = 3$, $P = 0.002$; and colony H: $H = 11.434$, $df = 3$, $P = 0.010$). Imidacloprid at 1.00 μg/ml significantly suppressed digging activity of ants from all tested colonies. For the number of dead ants, significant difference was found between imidacloprid at 1.00 μg/ml and control in two of the four colonies (Table 2, colony A: $F_{3,16} = 1.718$, $df = 19$, $P = 0.203$; colony B: $F_{3,16} = 2.701$, $df = 19$, $P = 0.080$; colony C: $H = 15.798$, $df = 3$, $P = 0.001$; colony D: $H = 13.715$, $df = 3$, $P = 0.003$). In experiment 2 (0.25, 0.05, and 0.01 μg/ml imidacloprid), digging activity was significantly reduced after ants had fed on 0.25 μg/ml imidacloprid in three of the four colonies (Table 2). Imidacloprid at 0.01 μg/ml significantly increased the digging than control in two of the four colonies. For number of dead ants, there was a significant difference between 0.25 μg/ml imidacloprid and control in three of the four colonies (Table 2, colony E: $H = 15.799$, $df = 3$, $P = 0.001$; colony F: $H = 7.164$, $df = 3$, $P = 0.067$; colony G: $F_{3,16} = 18.017$, $df = 19$, $P = 0.0001$; colony H: $F_{3,16} = 3.318$, $df = 19$, $P = 0.047$).

In Bioassay 2, ants in control but not imidacloprid treatments had limited sugar water supply before the digging bioassay. For experiment 1 (0.25, 0.50, and 1.00 μg/ml imidacloprid), sugar water consumption and imidacloprid ingestion are summarized in Supp. Figure 3A (online only). The amount of sugar water consumed was not significantly different among control, 0.25, and 0.50 μg/ml imidacloprid treatments, but fire ant consumed less sugar water containing 1.00 μg/ml imidacloprid (Supp. Fig. 3A [online only], $H = 8.029$, $df = 3$, $P = 0.045$). Amount of removed sand and ant mortality are summarized in Table 3. In two of the four colonies, after feeding on 1.00 μg/ml imidacloprid for 7 d, most ants died and there were not enough ants for the digging bioassays. There was a significant difference on the amount of removed sand among treatments and control for each colony (colony I: $H = 15.869$, $df = 3$, $P = 0.001$; colony J: $H = 15.937$, $df = 3$, $P = 0.001$; colony K: $H = 12.020$, $df = 2$, $P = 0.002$; colony L: $H = 12.522$, $df = 2$, $P = 0.002$; colony M: $F_{3,16} = 6.512$, $df = 19$, $P = 0.004$; colony N: $F_{3,16} = 8.000$, $df = 19$, $P = 0.002$; colony O: $F_{3,16} = 13.165$, $df = 19$, $P = 0.0001$; and colony P: $F_{3,16} = 6.149$, $df = 19$, $P = 0.006$). Imidacloprid at 1.00 μg/ml significantly suppressed digging activity in both tested colonies. For the number of dead ants, significant difference was found between imidacloprid at 1.00 μg/ml and control in all test colonies (Table 3, colony I: $H = 10.655$, $df = 3$, $P = 0.014$; colony J: $H = 14.849$, $df = 3$, $P = 0.002$; colony K: $F_{3,16} = 40.053$, $df = 19$, $P = 0.0001$; colony L: $F_{3,16} = 8.775$, $df = 19$, $P = 0.004$). The difference was not significant between imidacloprid at 0.25 μg/ml and control in all colonies. For experiment 2 (0.01, 0.05, and 0.25 μg/ml imidacloprid), the mean daily consumption rates of sugar water for the whole feeding period (7 d) are summarized in Supp. Figure 3B (online only) and the consumption rate for each day is shown in Supp. Figure 4 (online only). The consumption of sugar water was not significantly different between control and 0.25 μg/ml imidacloprid treatment in the first day ($t = -2.31$, $df = 3.983$, $P = 0.825$; Supp. Fig. 4 [online only]). However, in the second day, consumption of sugar water containing 0.25 μg/ml imidacloprid decreased significantly ($t = -2.31$, $df = 3.454$, $P = 0.017$; Supp. Fig. 4 [online only]). The daily sugar water consumption was not significantly different among control, 0.01, and 0.05 μg/ml imidacloprid; however, fire ant workers consumed less sugar water at 0.25 μg/ml imidacloprid (Supp. Fig. 3B [online only], $H = 6.904$, $df = 3$, $P = 0.075$). Amount of removed sand and ant mortality are summarized in Table 3. Digging behavior was significantly reduced after ants fed on 0.25 μg/ml imidacloprid sugar water in three of the

Table 2. Number of dead ants and amount of removed sand 24 h after 30 workers were released in digging bioassay apparatus ($n = 5$)

Colony	Concn (μg/ml)	Subcolony weight (g)	Sugar water consumed after 7 d (g)	Number of death (mean ± SEM)	Sand removed (mg, mean ± SEM)
A	0.00	8.0	14.1	0.4 ± 0.4a	1,774.34 ± 238.51a
	0.25	8.0	4.8	0.8 ± 0.4a	878.28 ± 163.49b
	0.50	8.0	2.0	1.4 ± 0.2a	321.40 ± 44.70c
	1.00	8.0	2.0	0.4 ± 0.4a	238.64 ± 125.41c
B	0.00	8.0	21.5	0.8 ± 0.5ab	1,501.54 ± 459.01a
	0.25	8.0	4.7	2.0 ± 0.4ab	856.54 ± 257.13a
	0.50	8.0	1.2	0.6 ± 0.2b	927.20 ± 125.49a
	1.00	8.0	2.8	2.4 ± 0.8a	73.48 ± 30.58b
C	0.00	8.0	34.6	0.4 ± 0.2b	5,362.20 ± 261.01a
	0.25	8.0	8.0	1.2 ± 0.5b	2,142.66 ± 211.06b
	0.50	8.0	5.1	7.4 ± 1.7a	106.32 ± 35.64c
	1.00	8.0	5.3	11.2 ± 0.7a	0.10 ± 0.10d
D	0.00	14.0	28.9	0.2 ± 0.2b	3,685.74 ± 224.64a
	0.25	14.0	6.2	0.2 ± 0.2b	1,348.50 ± 162.04b
	0.50	14.0	2.8	0.2 ± 0.2b	272.68 ± 66.54c
	1.00	14.0	3.3	4.6 ± 0.7a	105.22 ± 27.88c
E	0.00	2.5	2.3	2.6 ± 0.7c	1,405.34 ± 387.36a
	0.01	2.5	2.7	2.0 ± 0.6c	2,104.64 ± 334.17a
	0.05	2.5	2.7	6.6 ± 0.8b	242.60 ± 75.31b
	0.25	2.5	2.3	16.0 ± 2.4a	1.30 ± 0.66b
F	0.00	4.3	4.2	2.6 ± 0.2a	836.52 ± 203.67b
	0.01	4.3	8.0	1.4 ± 0.5a	1,999.64 ± 249.93a
	0.05	4.3	7.5	1.6 ± 0.5a	1,395.88 ± 224.90ab
	0.25	4.3	4.2	3.8 ± 0.7a	1,023.28 ± 136.66b
G	0.00	4.2	4.9	6.0 ± 0.4b	1,099.16 ± 234.22b
	0.01	4.2	6.4	2.6 ± 0.4c	3,066.88 ± 534.06a
	0.05	4.2	3.8	3.6 ± 1.1bc	3,303.68 ± 215.31a
	0.25	4.2	3.9	11.0 ± 1.2a	327.10 ± 172.63c
H	0.00	3.7	4.6	1.2 ± 0.7b	3,661.02 ± 223.62a
	0.01	3.7	5.4	2.2 ± 0.6ab	3,547.90 ± 413.72a
	0.05	3.7	5.0	1.8 ± 0.7b	3,212.26 ± 321.24a
	0.25	3.7	2.7	4.6 ± 1.2a	521.16 ± 155.52b

Ants had fed on sugar water with various concentrations of imidacloprid for 7 d, in which sugar water supply was not limited.

The LSD test or Mann–Whitney test was used to compare the amount of sand removed and number of dead workers among treatments and controls. Means with same letter were not significantly different ($P > 0.05$).

four colonies (Table 3). Imidacloprid at 0.05 μg/ml did not significantly increase the number of dead ants for all colonies (Table 3, colony M: $H = 9.568$, $df = 3$, $P = 0.023$; colony N: $H = 5.766$, $df = 3$, $P = 0.124$; colony O: $F_{3,16} = 3.944$, $df = 19$, $P = 0.028$; colony P: $H = 15.106$, $df = 3$, $P = 0.002$).

Foraging Bioassay. Mean daily imidacloprid intake per gram workers was 1.03 ng when colonies were exposed to 0.01 μg/ml imidacloprid and 18.31 ng when exposed to 0.25 μg/ml imidacloprid. Discovery time was not significantly affected by imidacloprid at both concentration (Fig. 2; 0.01 μg/ml: $t = -0.739$, $df = 8$, $P = 0.481$; 0.25 μg/ml: $t = 0.592$, $df = 5.452$, $P = 0.577$), and neither was the recruitment time (0.01 μg/ml: $t = 1.425$, $df = 8$, $P = 0.192$; 0.25 μg/ml: $t = -1.102$, $df = 4.010$, $P = 0.332$). At 0.01 μg/ml imidacloprid, ants in three colonies discovered baits and recruited nestmates within 20 min, and at 0.25 μg/ml imidacloprid, ants in four colonies discovered baits and recruited nestmates within 10 min.

Imidacloprid significantly reduced the percentage of ants shown up on the bait at both concentrations (Fig. 3), 0.01 μg/ml (Fig. 3A, $F_{1,6} = 43.47$, $df = 7$, $P < 0.0001$), and 0.25 μg/ml (Fig. 3B, $F_{1,6} = 159.58$, $df = 7$, $P < 0.0001$).

The time needed by fire ant workers to cross 20.0-cm horizontal segment of the bridge was not significantly affected by 0.01 μg/ml imidacloprid in three of the four colonies (Fig. 4A; colony 2: $U = 281.500$, $df = 1$, $P = 0.547$; colony 3: $U = 130.000$, $df = 1$, $P < 0.0001$; colony 4: $U = 232.000$, $df = 1$, $P = 0.118$; colony 5: $U = 245.000$, $df = 1$, $P = 0.190$). The results did not include colony 1, because there were not enough ants that cross the bridge after exposure to imidacloprid. The time required to cross the bridge increased significantly by exposure to 0.25 μg/ml imidacloprid in all test colonies (Fig. 4B; colony 6: $U = 185.500$, $df = 1$, $P = 0.014$; colony 7: $U = 66.000$, $df = 1$, $P < 0.0001$; colony 8: $U = 182.000$, $df = 1$, $P = 0.011$; colony 9: $U = 153.500$, $df = 1$, $P = 0.002$; colony 10: $U = 124.000$, $df = 1$, $P = 0.001$).

The amount of baits retrieved by workers was significantly affected by imidacloprid treatment (Fig. 5). Before exposure to 0.01 μg/ml imidacloprid, one gram of workers retrieved 96.96 mg baits; however, they only retrieved 12.50 mg baits after exposure to imidacloprid ($U = 0.000$, $df = 1$, $P = 0.0009$). Before exposure to 0.25 μg/ml imidacloprid, one gram workers retrieved 216.90 mg of baits; however, after exposure to imidacloprid, only 1.38 mg bait was retrieved by per gram workers ($U = 0.000$, $df = 1$, $P = 0.0009$). There was no significant difference in daily weight loss of ant between control and imidaclporid exposure at 0.01 μg/ml ($t = 0.922$, $df = 8$, $P = 0.384$) and 0.25 μg/ml ($t = 0.594$, $df = 8$, $P = 0.569$; Supp. Fig. 5 [online only]).

Table 3. Number of dead ants and amount of removed sand 24 h after 30 workers were released in digging bioassay apparatus ($n = 5$)

Colony	Concn (μg/ml)	Subcolony weight (g)	Sugar water consumed after 7 d (g)	No. of dead ants (mean ± SEM)	Sand removed (mg, mean ± SEM)
I	0.00	6.3	6.7	4.4 ± 1.7ab	2,444.90 ± 198.50a
	0.25	6.3	8.3	1.4 ± 0.2b	2,163.78 ± 379.43a
	0.50	6.3	6.6	1.0 ± 0.4b	329.88 ± 76.96b
	1.00	6.3	3.7	8.0 ± 1.9a	20.40 ± 9.02c
J	0.00	2.6	2.3	3.8 ± 0.9b	1,807.64 ± 86.26a
	0.25	2.6	3.5	1.6 ± 0.2c	986.46 ± 277.29b
	0.50	2.6	2.4	4.4 ± 0.9b	320.38 ± 75.72b
	1.00	2.6	2.0	10.8 ± 0.7a	37.92 ± 22.43d
K	0.00	3.0	2.5	3.6 ± 0.7b	1,094.42 ± 108.30a
	0.25	3.0	2.7	4.2 ± 0.8b	578.44 ± 109.34b
	0.50	3.0	2.2	13.4 ± 1.1a	9.44 ± 3.55c
	1.00	3.0	1.7	–	–
L	0.00	5.0	5.3	3.2 ± 0.7b	2,112.72 ± 234.82a
	0.25	5.0	3.8	10.0 ± 0.7a	143.28 ± 48.90b
	0.50	5.0	4.0	15.2 ± 3.4a	2.90 ± 1.62c
	1.00	5.0	2.0	–	–
M	0.00	6.1	10.1	12.0 ± 4.1a	1,527.80 ± 332.44ab
	0.01	6.1	9.1	2.0 ± 1.0b	1,855.86 ± 197.12a
	0.05	6.1	8.1	3.8 ± 1.2ab	951.44 ± 223.58bc
	0.25	6.1	4.3	1.0 ± 0.4b	534.96 ± 116.92c
N	0.00	5.5	4.5	1.4 ± 0.7a	1,807.64 ± 131.52a
	0.01	5.5	5.2	0.4 ± 0.2a	1,575.12 ± 182.81a
	0.05	5.5	2.7	0.0 ± 0.0a	1,286.32 ± 198.71a
	0.25	5.5	2.8	4.4 ± 2.0a	566.36 ± 234.62b
O	0.00	4.0	7.7	1.2 ± 1.3ab	1,626.06 ± 302.87a
	0.01	4.0	6.2	0.6 ± 0.5b	1,657.98 ± 397.21a
	0.05	4.0	6.9	0.4 ± 0.5b	2,305.70 ± 163.01a
	0.25	4.0	2.8	2.8 ± 1.9a	60.82 ± 21.79b
P	0.00	7.0	8.0	0.2 ± 0.4b	1,938.62 ± 147.59b
	0.01	7.0	8.1	3.8 ± 1.8a	2,933.00 ± 325.65a
	0.05	7.0	5.4	0.6 ± 0.5b	1,618.70 ± 367.38b
	0.25	7.0	5.1	2.2 ± 1.1a	1,310.74 ± 243.20b

Ants had fed on sugar water with various concentrations of imidacloprid for 7 d, in which sugar water supply in control (0.00 μg/ml imidacloprid) was limited.

The LSD test or Mann–Whitney test was used to compare the amount of sand removed and number of dead ants among treatments and controls. Means with same letter were not significantly different ($P > 0.05$). In colonies K and L, after feeding on 1.00 μg/ml imidacloprid for 7 d, most ants died and there were not enough ants for the digging bioassays.

Discussion

Imidacloprid has antifeedant effects on several insects even at very low concentrations, for instance, 0.006 μg/ml for peach-potato aphid (Devine et al. 1996), 0.375 μg/ml for termite (Ramakrishnan et al. 2000), and 0.001 μg/ml for Asian citrus psyllid (Boina et al. 2009). In this study, it was found that imidacloprid at 0.25, 0.50, and 1.00 μg/ml reduced the feeding of *S. invita*. It was suggested that Bumble bee-reduced feeding on syrup containing imidacloprid was due to imidacloprid toxicity rather than aversion (Laycock et al. 2012). In this study, the consumption of sugar water was not significantly different between control and 0.25 μg/ml imidacloprid treatment in the first day, but was significantly lower in the second day, suggesting that the reduced feeding might be due to imidacloprid toxicity rather than aversion. Imidacloprid at 0.05 and 0.01 μg/ml did not inhibit *S. invicta* feeding. In contrast, fire ant workers even consumed more sugar water containing 0.01 μg/ml imidacloprid than the untreated sugar water. Researches have shown that sublethal concentrations of neurotoxic insecticides stimulated excessive feeding of some insect species (O'Brien et al. 1985, Haynes 1988). For example, at low doses, chlordimeform stimulated hypephagia, but at higher sublethal doses it caused anorexia (Pfister et al. 1978). Imidacloprid may have the similar effect on fire ants. When fire ants have choices (untreated sugar water vs. 0.01 μg/ml imidacloprid sugar water), consumption was not significantly different between untreated sugar water and imidacloprid-treated sugar water, indicating that fire ants did not have feeding preference toward sugar water containing imidacloprid.

Imidacloprid at ≥0.25 μg/ml significantly reduced fire ant digging activity. In contrast, in two of the four colonies, ants fed with 0.01 μg/ml imidacloprid significantly increased digging activity. Digging is an important behavior for subterranean ant species, which is involved not only in building, enlarging, and repairing nest but also in constructing foraging tunnels. Interfering with digging behavior may have a great impact on the ant colony. Sublethal concentrations of imidacloprid were reported to suppress *Reticulitermes virginicus* tunneling behavior (Thorne and Breisch 2001). Digging behavior may be a sensitive indicator in assessing the sublethal effect of toxins on social insects that exhibit digging behavior.

In foraging bioassay, it was found that exposure to 0.25 μg/ml imidacloprid did not alter the food discovery time and recruitment time of *S. invicta*. This finding is consistent with previous study, which showed that exposure of Southern ants, *M. antarcticum*, and

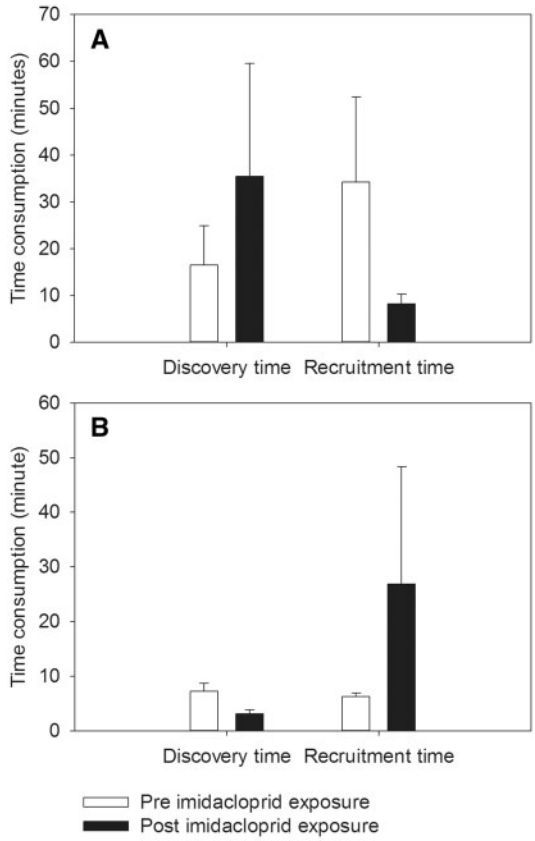

Fig. 2. Mean (± SE) food discovery time and recruitment time of fire ant workers pre and post imidacloprid treatments at 0.01 µg/ml (A) and 0.25 µg/ml (B). There was no significant difference (*t*-test, $P > 0.05$). For each treatment, $n = 5$.

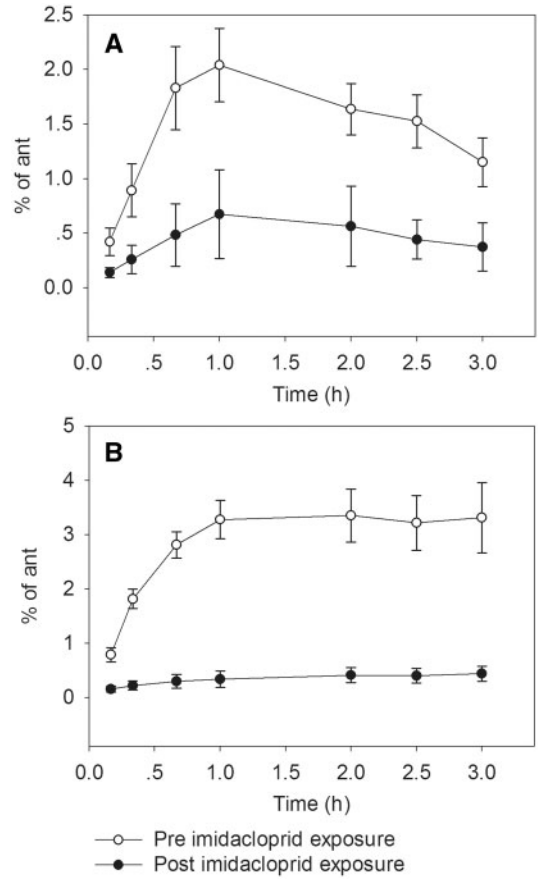

Fig. 3. Mean percentage (± SE) of ants shown up on the bait during timed observations pre and post imidacloprid treatments at 0.01 µg/ml (A) and 0.25 µg/ml (B). For each treatment, $n = 5$.

Argentine ants, *L. humile*, to 1.0 µg/ml imidacloprid didn't affect food discovery (Barbieri et al. 2013). However, the number of fire ant on baits and retrieval rate were decreased significantly after imidacloprid exposure. The walking speed of workers were also significantly reduced by feeding sugar water containing 0.25 µg/ml imidacloprid. Intuitively, high walk speed should facilitate food finding. Why were discovery time and recruitment time not affected, even when walking speed had been significantly reduced? Finding a food is a complicated process and the losing speed may have been compensated by some unknown factors that can be enhanced by low level of imidacloprid. One possibility is that ants fed on low level of imidacloprid may be more sensitive to chemical cues involved in food finding and recruitment. This should be a venue for further research.

There was no significant difference in ant daily mortality between control and imidaclporid exposure at 0.01 and 0.25 µg/ml. In a study over 8 wk, imidacloprid at 0.006 µg/ml showed negative impact on bumble bee population and queen production (Whitehorn et al. 2012). In this study, ants were fed with imidacloprid-containing sugar water for only 7 d. For understanding chronic effects of pesticides to pollinators, Rondeau et al. (2014) constructed a time-to-effect measurement based on published toxicity data of imidacloprid for several insects. It was suggested the chronic effect test should be extended to 30 d or more for pollinators (Rondeau et al. 2014). It will be interesting to see whether long-term intake of 0.01 µg/ml imidaclporid (>7 d) causes increase of *S. invicta* mortality at colony and population level.

Imidacloprid at 0.01 µg/ml increased the sugar water consumption and digging activity. Both feeding and digging behaviors can have a significant effect on the efficacy of insecticides. For example, an ideal active ingredient of fire ant bait should not deter the feeding of fire ants, so it should not be a repellant (Stringer et al. 1964, Williams et al. 2001). Delayed suppression on the social behaviors, such as digging behavior, may enhance the efficacy of imidacloprid on termites (Henderson 2003). If feeding on low dose imidacloprid for an extended period of time has a chronic effect on fire ants, it may be useful as a fire ant bait active ingredient. In fire ant mound treatment, efficacy of

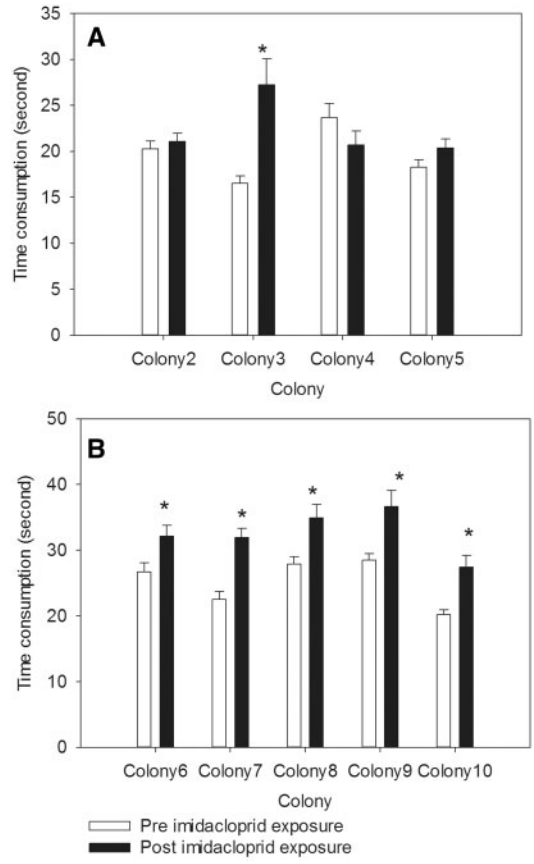

Fig. 4. Average time (± SE) needed by fire ant workers to cross 20-cm segment of the bridge pre and post imidacloprid treatments at 0.01 μg/ml (A) and 0.25 μg/ml (B). *: significant difference between pre- and post-imidacloprid treatments (*t*-test, $P < 0.05$). For each treatment, $n = 25$.

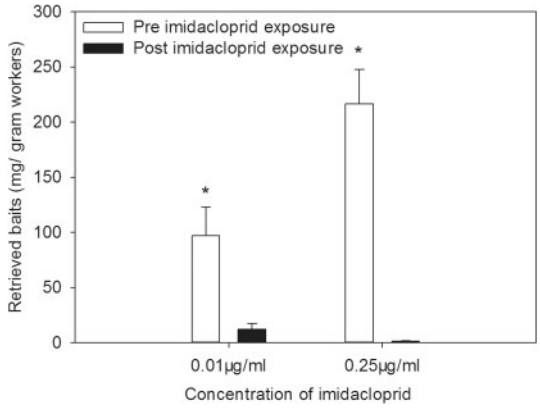

Fig. 5. Mean bait retrieval rate (± SE) pre and post imidacloprid treatments. *: significant difference between pre- and post-imidacloprid treatments (*t*-test, $P < 0.05$). For each treatment, $n = 5$.

imidacloprid may be improved by using right concentrations that have delayed effect on the ant digging behavior. This study indicates potential for more effective utilization of imidacloprid in fire ant management. However, on the other hand, it also raises an alert for the potential detrimental impact of imidacloprid residuals on the beneficial ants.

Supplementary Data

Supplementary data are available at *Environmental Entomology* online.

Acknowledgments

We thank Dr. Abbas Ali, School of Pharmacy, The University of Mississippi, Oxford, MS, and Dr. Beverly Wiltz, Delgado Community College, New Orleans, LA, for critical reviews of the manuscript. We thank Mr. Leon Hicks and Ms. Weihua Jiang for their technical assistance. This project is supported by the Hesheng Zhujiang Education Foundation, South China Agricultural University and the Post-graduates Study Abroad Program, China Scholarship Council.

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Received 21 May 2015; accepted 20 July 2015.