Red Imported Fire Ants (Hymenoptera: Formicidae) Cover the Insecticide-Treated Surfaces with Particles to Reduce Contact Toxicity

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Research Article

Keywords: *Solenopsis invicta*, Insecticide resistance, Particle-covering behavior, Repellency, Tool use

Posted Date: October 1st, 2021

DOI: <https://doi.org/10.21203/rs.3.rs-821548/v1>

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Abstract

Surface treatment is commonly used in controlling the red imported fire ants, *Solenopsis invicta* Buren. In the present study, the behavioral responses of *S. invicta* workers to surfaces treated with insecticides were investigated. Toxicological tests showed that beta-cypermethrin had the highest contact toxicity (with the lowest LC_{50} value) among nine tested insecticides, followed by thiamethoxam, fipronil, indoxacarb, chlorfenapyr, rotenone, spinetoram, avermectin, and chlorantraniliprole. In the laboratory, surfaces treated beta-cypermethrin or rotenone significantly reduced the number of foraging ants. In addition, *S. invicta* workers transported significantly more particles (measured in weight and/or covered area) onto surfaces treated with fipronil (50, 500, and 5000 ppm), rotenone (5000 ppm), or avermectin (5000 ppm) compared with the controls. Similarly, these insecticides significantly triggered the particle-covering behavior of ants in the field. We hypothesized that such behaviors would reduce the contact toxicity of insecticides against *S. invicta*. When the surfaces treated with fipronil or rotenone (500 or 5000 ppm) were artificially covered with particles, *S. invicta* had significantly higher LT_{50} values compared with insecticide-treated surfaces without particles. This study provides the first evidence that *S. invicta* workers can perform particle-covering behavior to reduce the toxicity of certain insecticides, which constitutes a unique insecticide-resistance strategy in ants.

Key Message

Surface treatment of nine insecticides had concentration-dependent toxicity against *S. invicta* workers.

S. invicta workers relocated significantly more soil particles to cover the surfaces treated with fipronil, rotenone, or avermectin than untreated surfaces.

Using particles to artificially cover the surfaces previously treated with fipronil or rotenone significantly decreased the toxicity of these insecticides against *S. invicta* workers

Introduction

The red imported fire ant, *Solenopsis invicta* Buren, is a significant urban pest that stings people and causes severe symptoms and even death (Vinson 2013; Wang et al. 2020). This species is also an important agricultural pest because it can damage various crops and livestock (Vinson 2013). *S. invicta* also competes with native ant species and prey on many invertebrates and vertebrates, posing great threats to the biodiversity of invaded ecosystems (Vinson 2013). Unfortunately, *S. invicta* has kept spreading (Ascunce et al. 2011). For example, *S. invicta* colonies have been reported in 390 counties of 13 provinces in China since 2024, just 16 years after it was first found in Wuchuan, Guangdong, China (Wang et al. 2020).

Two methods have been widely applied to control *S. invicta*. One is using the oil-based baits containing slow-acting oral toxicants (Oi 2006; Wang et al. 2020). Another method relies on the contact-based insecticides that can be used in mound and surfaces treatments (Rust and Su 2010). The contact-based insecticides can be either fast-acting that cause the rapid mortality of *S. invicta*, or slow-acting that provide ants with enough time and chance to horizontally transfer the insecticide among nestmates. Chen and Oi (2020) reviewed the synthetic chemicals or naturally-occurring compounds that can be used as contact-based insecticides against *S. invicta*. Over 30 products have been registered in US for *S. invicta* control.

Knowledge in the biology of the targeted insects is always critical to the success of any insect pest management programs. For contact-based fire ant control products, tremendous effort has been made in studying the nature of the insecticide (e.g., toxicity and mode of action) and developing new formulations and application methods; however, less attention has been paid to the effect of ant behaviors on the insecticide efficacy. Our previous studies showed that *S. invicta* workers usually cover the wet, sticky, or repellent surfaces with soil particles, and they can walk on these particles to avoid direct contacting the inaccessible surfaces (Wang and Henderson 2016; Wen et al. 2016, 2020a, b). These behaviors have provoked our interest in investigating whether *S. invicta* workers would cover the insecticide treated surfaces with soil particles to avoid the contact with insecticides, and therefore reduce the effectiveness of the insecticides.

Herein, we hypothesized that (1) *S. invicta* workers cover surfaces treated with contact-based insecticides, and (2) such behavior reduces the contact toxicity of insecticides. In the present study, toxicity and behavioral effects of nine insecticides (beta-cypermethrin, thiamethoxam, fipronil, indoxacarb, chlorfenapyr, rotenone, spinetoram, avermectin, and chlorantraniliprole) on *S. invicta* workers were investigated (Table 1). Beta-cypermethrin, fipronil, and rotenone have been applied as contact-based insecticides to eliminate *S. invicta* populations (Chen et al. 2006; Chen and Oi 2020). The potential of thiamethoxam and chlorfenapyr as contact-based insecticides against *S. invicta* has been confirmed by Wiltz et al. (2010). Although indoxacarb and avermectin have high oral toxicity against *S. invicta* (Williams 1985; Chen and Oi 2020), their contact toxicity has not yet been reported. We also evaluated two widely-applied insecticides, spinetoram and chlorantraniliprole, for their lethal and behavioral effects on *S. invicta*.

Table 1

Common name, commercial name, percentage of active ingredient (a.i.), manufacturer, and mode of action of insecticides used in the present study.

Insecticide name	Commercial name	Active ingredient (a.i. %)	Manufacturer	Mode of action
Beta-cypermethrin	Zhb® EC	4.5	Hebei Zhongbao Green Crops Technology Co., Ltd., China	Sodium channel modulator (Horia <i>et al.</i> , 2001; Linda <i>et al.</i> , 2017; IRAC, 2019)
Thiamethoxam	Zhongbaoruishi® EC	30	Hebei Zhongbao Green Crops Technology Co., Ltd., China.	Nicotinic acetylcholine receptor (nAChR) competitive modulators (IRAC, 2019).
Fipronil	Termidor® EC	2.5	Bayer China Co., Ltd., Shanghai, China	Gamma-aminobutyric acid (GABA) gated chloride channel blocker (Cole <i>et al.</i> , 1993; Hosie <i>et al.</i> , 1995; IRAC, 2019)
Indoxacarb	Kaien® SC	15	FMS (China) Investment Co., Ltd. Shanghai, China	Voltage-dependent sodium channel blocker (Jun <i>et al.</i> , 2002; Li <i>et al.</i> , 2013; IRAC, 2019)
Chlorfenapyr	Jialvyuan® EC	10	BISSELL, Henan Agricultural Science and Technology Co., Ltd., China	Uncouplers of oxidative phosphorylation via disruption of proton gradient (IRAC, 2019)
Rotenone	Baiyang® MG	6	Beijing Multigrass Formulation Co., Ltd., China.	Mitochondrial complex I electron transport inhibitors
Spinetoram	Ailvshi® SC	6	Dow AgroSciences (China) Co., Ltd., Shanghai, China	Nicotinic acetylcholine receptor (nAChR) allosteric modulator (Zhang, 2014; IRAC, 2019)
Avermectin	Zhongbaojinhu® EC	5	Hebei Zhongbao Green Crops Technology Co., Ltd., China.	Glutamate-gated chloride channel (GluCl) allosteric modulators. (IRAC, 2019).
Chlorantraniliprole	Coragen® EC	20	FMS (China) Investment Co., Ltd. Shanghai, China	Ryanodine receptor modulators (IRAC, 2019).

Materials And Methods

Toxicity of insecticide-Treated Surfaces against *Solenopsis invicta*

Ant Collection and Maintenance

Six colony groups of *S. invicta* were collected from the Tianlu Lake Park (23°23' N, 113°41' E), Guangzhou, China, on 7 October 2020. Right before collecting, the ethanol-talc mixture (3% [w/w]) was prepared using the method provided by Ning *et al.* (2019), which was evenly smeared on the inner wall of a plastic box (50 × 37 × 26 cm [L × W × H]) to prevent ant escaping. A *S. invicta* mound was randomly selected, and nest materials (20–30 L) containing large amounts of eggs, larvae, pupae, and adults of ants were rapidly transferred to the plastic box using a shovel. The boxes were brought back to the lab within 2 h after collection. Ants were fed with 20% honey-water solution and tap water, and frozen crickets were regularly provided. Colonies were maintained with a photoperiod of 16 : 8 (L : D) h at room temperature (20–28°C).

Toxicological Bioassays

This study aimed to investigate the contact toxicity of insecticides against *S. invicta* workers. Only workers were used in the bioassays. Nine commercial formulations of insecticides were tested in separate experiments. The bioassay arenas were beakers (bottom diameter: 10.4 cm; height: 14.0 cm; Bomei Glass Instrument Factory, Beijing, China) with the inner wall coated with Teflon (500 mL, GUOXIN®, Dongguan, China). For each insecticide, solutions with different concentrations of the active ingredient (0.05, 0.5, 5, 50, 500, and 5000 ppm) were prepared by dissolving the required amount of the insecticide in distilled water, and 170 µL of the solution was added and evenly smeared on the bottom of the beaker (to reach the final amount of 2 µL/cm²). The same amount of distilled water was smeared on the bottom of the beaker as the controls. These beakers were air-dried at room temperature (22 ± 2°C) for 24 h. Right before the experiment, a sterile L-shaped cell spreader (Biglogix 65-1010, BIOLOGIX®, Jinan, China) was inserted into the plastic box containing each *S. invicta* colony, and workers were allowed to climb up on the surface of the pipette, which was then inserted into the beaker (~ 5 cm above the bottom) and gently shaken until 20 medium workers fall onto the bottom of the beaker. Each concentration of each insecticide was repeated 6 times (one replicate for each colony group). To prevent dehydration of ants, the beakers were placed in plastic boxes with 3 layers of paper towel (moistened using 200 mL distilled water) placed on the bottom, and the plastic boxes were capped to maintain the humidity (Mao *et al.* 2011). All laboratory experiments were conducted at room temperature (22 ± 2°C). The mortality of ants was recorded at 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 36, 48, 60, 72 h.

Behavioral Effects of Insecticide-Treated Surfaces on *Solenopsis invicta* Under Laboratory Conditions

This study aimed to investigate the foraging and particle-covering behaviors of *S. invicta* workers in response to surfaces treated with insecticides under laboratory conditions. Seventy-two colony groups of *S. invicta* were used in this experiment. Eight colony groups were collected from the campus of South China Agricultural University (23°17'N, 113°37'E), 32 colony groups were collected from the Zengcheng Teaching and Internship Base of South China Agricultural University (23° 24' N, 113° 63' E), and the remained colony groups were collected from Tianlu Lake Park. Nest materials (15–20 L) and ants were transferred to plastic boxes (44 × 29 × 19 cm [L × W × H]) using the similar methods described earlier. Ants were maintained in the laboratory for ~ 1 week before the experiment.

The test for each insecticide was repeated 8 times, and each colony group was tested only once. Before the test, solutions with different concentrations of the active ingredient (0.05, 0.5, 5, 50, 500, and 5000 ppm) were prepared as described above. Plastic squares (50 × 50 mm) were prepared by coating a piece of white cardboard paper with a layer of plastic membrane (Qin et al. 2019), and 50 µL solution was evenly smeared onto the square (the final amount of the solution on each square was 2 µL/cm²). To prepare the untreated (control) squares, the same amount of distilled water was added onto the square and evenly smeared. These squares were air-dried for ~ 8 h. Right before the test, a piece of sausage (10 × 10 × 1 mm, Guangdong Shuanghui Food Co. Ltd., Qingyuan, China) was fixed onto the center of each square using an insect pin (Length = 35 mm, diameter = 0.35 mm). Seven squares either smeared with the active ingredient (0.05, 0.5, 5, 50, 500, or 5000 ppm) or distilled water were placed on the plastic box with randomly assigned orders, and adjacent squares were > 1 cm apart from each other (Fig. 1A).

High-resolution pictures were taken for each square at 15, 30, 45, 60 min, and 3, 6, 12, 24 h into the experiment. The number of ants on each square was counted at 15, 30, 45, and 60 min, or until the food was completely transported away from the square within 1 h. The data obtained from different time points were averaged to determine the foraging activities of ants within 1 h (Wen et al. 2020a). The presence of sausages on each square was recorded at 1, 3, 6, 12, and 24 h. Pictures taken at the end of the experiment (24 h) were analyzed to measure the area of squares covered by particles using the regionprops function of MATLAB (MathWorks Inc., Natick, MA, USA). Also, particles on each square were collected and oven-dried at 50°C for 5 d. The dry weight of particles was measured using a 0.1-mg electronic balance (Mettler Toledo®, Switzerland).

Behavioral Effects of Insecticide-Treated Surfaces on *Solenopsis invicta* Under Field Conditions

This study aimed to investigate the effect of insecticide-treated surfaces on the foraging and particle-covering behaviors of *S. invicta* workers under field conditions. The experiments were conducted in Zengcheng Teaching and Internship Base of South China Agricultural University from 19–23 April 2021. Twenty *S. invicta* mounds were randomly selected, and weeds and rocks around mounds were removed 4 d before the experiments. Solution of each insecticide (500 or 5000 ppm) was prepared, and 50 µL solution was evenly smeared onto the square. For each concentration (500 or 5000 ppm), squares either treated with insecticide or distilled water were placed 20-cm apart from the edge of the *S. invicta* mound (Fig. 1B). The order and direction of squares were randomly assigned, and adjacent squares were equidistant from each other. Each concentration was repeated 10 times (each mound was tested only once). The average number of foraging ants within 1 h, the presence of food at 1, 3, 6, and 24 h, and dry weight and covered areas of relocated particles at the end of the experiment (24 h) were recorded as described above.

Effect of Particle-Covering Behavior on Toxicity of Contact-Based Insecticides against *Solenopsis invicta*

Our laboratory and field study showed that *S. invicta* workers transported significantly more particles onto the squares smeared with fipronil or rotenone compared with the control ones (see results). Herein, we conducted a laboratory study to investigate whether such particle-covering behaviors would reduce the toxicity of fipronil or rotenone against *S. invicta*.

Six colony groups of *S. invicta* were collected from Tianlu Lake Park on 24 February 2021, using the same method as described above. Topsoil was collected from the Zengcheng Teaching and Internship Base of South China Agricultural University where *S. invicta* activities were detected. The soil was oven-dried at 50°C for > 5 d. The dried soil was crushed using a wooden hammer and sequentially sifted through 0.5-mm and 1-mm sieves (our preliminary study showed that the diameters of most particles relocated by *S. invicta* workers were ranging from 0.5–1 mm). Before experiments, the required amount of distilled water was added into the soil and thoroughly mixed to prepare the 10%-moisture (w/w) particles.

The experiment for each insecticide contained 7 treatments (Table 2), and each treatment was repeated 6 times (one replicates for each colony group). One hundred and seventy microlitres of insecticide solution (500 or 5000 ppm) or distilled water was evenly smeared on the bottom of beakers with the inner wall coated with Teflon, and air-dried for > 12 h. Certain amounts of soil particles (Table 2) were evenly added onto the bottom of beakers (the density of particles was equal to the mean density of particles relocated onto the squares treated with fipronil or rotenone at the concentration of 500 or 5000 ppm in the laboratory study). Twenty medium *S. invicta* workers were released into each beaker and maintained in the plastic boxes with moistened paper towels as described earlier. The mortality of ants was recorded at 1, 2, 3, 4, 5, 6, 7, 8, 12, 24, 36, 48, 60, and 72 h for rotenone. Since all ants exposed to fipronil-treated surface (whether covered by particles or not) died within 12 h, the mortality of ants was no longer recorded after that time point.

Table 2

Experiment design to investigate whether particle-covering behavior reduces the contact toxicity of fipronil or rotenone against *Solenopsis invicta* workers.

Insecticide	Treatment	Concentration of insecticide solution smeared on the bottom of beakers (ppm)	Soil particles added onto the bottom of beakers (g)
Fipronil	0	0	
	0	4.44	
	3	0	5.26
	4	500	0
	5	500	4.44
	6	5000	0
	7	5000	5.26
Rotenone	1	0	0
	2	0	5.48
	3	0	8.98
	4	500	0
	5	500	5.48
	6	5000	0
	7	5000	8.98

Statistical Analyses

For experiments 2.1, the lethal concentration of *S. invicta* workers to each insecticide was calculated at the end of the experiment using the probit analysis (SAS 9.4, SAS Institute, Cary, NC). Because the data obtained from experiment 2.2–2.4 was not normal, nonparametric tests were used to analyze the data. For experiment 2.2, data (i.e., average number of foraging ants within 1 h, and dry weight and covered area of particles relocated by ants at the end of the experiment) were tested using nonparametric rank-based methods for mixed model (“nparLD” package in R [Noguchi et al. 2012]) with insecticide as the between-subject effect and concentration as the within-subject effect, and Dunn's Test with Bonferroni adjustments were performed for multiple comparisons. Since the effect of insecticide and concentration and their combination effect were significant (Supp. Materials: Table S1), different concentrations of each insecticide were compared separately. For experiment 2.3, data were tested using Kruskal-Wallis test (SAS 9.4), followed by Dunn's Test for multiple comparisons. For experiment 2.4, nonparametric rank-based methods for mixed model were used to analyze data with treatment as the between-subject factor, and time as the within-subject factor (“nparLD” package in R), followed by Dunn's Tests for multiple comparisons. Since the effect of treatment and time and their combination effect were significant (Supp. Materials: Table S1), data at each time point was compared separately. The significance level was determined at $\alpha = 0.05$ for all tests.

Result

Toxicity of Insecticide-Treated Surfaces against *Solenopsis invicta*

Beta-cypermethrin (0.5, 5, 50, 500, and 5000 ppm), thiamethoxam (50, 500, and 5000 ppm), fipronil (5, 50, 500, and 5000 ppm), indoxacarb (50, 500, and 5000 ppm), chlorfenapyr (500 and 5000 ppm), and rotenone (500 and 5000 ppm) caused 100% mortality of *S. invicta* workers at 72 h (Fig. 2A, B, C, D, E and F). However, spinetoram, avermectin, and chlorantraniliprole did not kill all ants even at the highest concentration (Fig. 2G, H and I). Among the nine tested insecticides, beta-cypermethrin had the highest contact toxicity (with the lowest LC_{50} value at 72 h), followed by thiamethoxam, fipronil, indoxacarb, chlorfenapyr, rotenone, spinetoram, and avermectin (Table 3). The LC_{50} value for chlorantraniliprole was not calculated because it caused < 50% mortality at all tested concentrations at the end of the experiment.

Table 3
Medium lethal concentrations (ppm) of each insecticide against *Solenopsis invicta* workers in the toxicological bioassays at 72 h.

Pesticides	n	LC ₅₀ (95% CL)	Regression equation	χ^2 (df)
Beta-cyhalothrin	120	0.062 (0.048-0.068)	$y = 4.054 - 3.360x$	38.590 (34)
Thiamethoxam	120	0.113 (0.033-0.259)	$y = 1.242 - 1.312x$	123.325 (34)
Fipronil	120	0.622 (0.384-1.005)	$y = 0.583 - 2.828x$	109.824 (34)
Indoxacarb	120	2.368 (1.095-4.988)	$y = -0.690 - 1.842x$	171.310 (34)
Chlorfenapyr	120	6.847 (3.015-15.337)	$y = -1.366 - 1.635x$	177.114 (34)
Rotenone	120	8.742 (2.734-28.503)	$y = -1.504 - 1.597x$	300.690 (34)
Spinetoram	120	8.637 (1.902-35.185)	$y = -0.670 - 0.716x$	189.302 (34)
Avermectin	120	57.124 (6.097-1469.470)	$y = -0.587 + 0.334x$	131.952 (34)

Behavioral Effects of Insecticides on *Solenopsis invicta* Under Laboratory Conditions

Within 1 h, significantly less foraging ants were found on the squares smeared with beta-cypermethrin (5, 50, 500, or 5000 ppm), rotenone (0.05, 5, 50, or 5000 ppm), or spinetoram (500 ppm) compared with the untreated squares (Fig. 3; **statistical results are shown in Supp. Materials: Table S2**). Thiamethoxam (0.05, 0.5, 5, or 5000 ppm), fipronil (0.05 or 0.5 ppm), and chlorfenapyr (5 ppm) significantly increased the number of foraging ants (Fig. 3). The number of foraging ants was not significantly different among untreated squares and squares smeared with different concentrations of indoxacarb, avermectin, or chlorantraniliprole (Fig. 3).

At the end of the experiment (24 h), few food items that placed on the squares smeared with beta-cypermethrin (500 or 5000 ppm), thiamethoxam (5000 ppm), or fipronil (5000 ppm) were transported by ants (Fig. 4). Significantly more particles (measured in both weight and covered area) were found on squares smeared with rotenone (5000 ppm) or avermectin (5000 ppm) compared with untreated squares (Figs. 5 and 6; **statistical results are shown in Supp.**

Materials: Table S3 and S4). In addition, significantly heavier particles were found on the squares smeared with fipronil (50, 500, or 5000 ppm) compared with the untreated squares (Fig. 5), but the particle-covered areas on fipronil-treated and untreated squares were similar (Fig. 6). The weight and covered-area of particles on the squares smeared with 5000-ppm indoxacarb were significantly greater than squares smeared with 50-ppm indoxacarb, but they were not significantly different from the untreated squares (Fig. 5, 6). Ants also relocated significantly heavier particles onto the squares smeared with 5000-ppm spinetoram than that smeared with 0.05-ppm spinetoram, but they were not different from the untreated squares (Fig. 5). No difference in particle weight and covered areas were detected among untreated squares and squares treated with different concentrations of beta-cypermethrin, thiamethoxam, chlorfenapyr, or chlorantraniliprole (Figs. 5, 6).

Behavioral Effects of Insecticides on *Solenopsis invicta* Under Field Conditions

At the concentration of 500 ppm, beta-cypermethrin and fipronil significantly reduced the number of foraging ants within 1 h (Fig. 7). No food placed on the square treated with beta-cypermethrin was transported by ants at the end of the experiment, whereas food on other squares was actively foraged by ants (Table 4). In addition, ants relocated significantly heavier particles onto the squares smeared with fipronil, rotenone, or avermectin than the untreated squares (controls), but only fipronil caused significantly more particle-covered area compared with the controls (Fig. 7).

Table 4
Number of food items that were not transported by *Solenopsis invicta* workers at each time point under field conditions. Each test was repeated 10 times. Bc indicate that most food (> 8 of 10 food items) was not transported.

Test	Time	Beta-cypermethrin	Thiamethoxam	Fipronil	Indoxacarb	Chlorfenapyr	Rotenone	Spinetoram	Avermectin	Chlorantraniliprole
500 ppm	1 h	10	10	10	10	9	10	9	10	10
	3 h	10	10	10	9	8	9	8	10	8
	6 h	10	1	10	8	6	8	8	10	8
	24 h	10	0	4	0	0	0	0	4	0
5000 ppm	1 h	10	10	10	10	10	10	9	10	9
	3 h	10	8	10	8	10	10	8	10	10
	6 h	10	8	10	8	10	10	7	10	5
	24 h	10	1	9	0	0	2	2	7	0

At the concentration of 5000 ppm, beta-cypermethrin, fipronil, chlorfenapyr, and rotenone significantly reduced the number of foraging ants within 1 h (Fig. 7). At 24 h, few food items on the square treated with beta-cypermethrin or fipronil were transported by ants (Table 4). Ants relocated more particles (measured in

both weight and covered area) onto the squares smeared with rotenone compared with the controls, whereas beta-cypermethrin significantly decreased the particle weight and covered area (Fig. 7).

Effect of Particle-Covering Behavior on Toxicity of Contact-Based Insecticides against *Solenopsis invicta*

All ants died within 5 h when they were exposed to the surfaces smeared with rotenone (500 or 5000 ppm) and when no particle was added (Fig. 8A). However, when rotenone-treated surfaces were artificially covered by particles, < 50% ants died at the end of the experiment (72 h). In addition, ants had significantly higher mortality when exposed to uncovered surfaces smeared with 5000-ppm fipronil than those artificially covered by particles within 5 h (Fig. 8B; **statistical results are shown in Supp. Materials: Table S5**). Covering the surfaces treated with 500-ppm fipronil also significantly decreased mortality within 8 h (Fig. 8B). For both insecticides and both concentrations, ants exposed to the treated surfaces artificially covered with particles had significantly longer medium lethal time (LT₅₀) compared with uncovered ones (Table 5).

Table 5
Medium lethal time (h) of each insecticide against *Solenopsis invicta* workers when the treated surfaces were artificially covered by soil particles or not.

Treatment	n	LT ₅₀ (95% CL)	Regression equation	χ ² (df)
Rotenone (5000 ppm + 0 g soil particles)	160	1.265 (1.148-1.393)	y = -0.26522.601x	176.665 (110)
Rotenone (5000 ppm + 8.98 g soil particles)	160	0.021 (0.004-0.122)	y = 1.4380.857x	67.658 (110)
Rotenone (500 ppm+ 0 g soil particles)	160	117.286 (76.350-180.169)	y = -5.6082.710x	73.561 (110)
Rotenone (500 ppm + 5.48 g soil particles)	160	185.731 (154.404-223.407)	y = -2.4931.099x	203.555 (110)
Fipronil (5000 ppm+ 0 g soil particles)	160	2.154 (2.037-2.778)	y = -18905.671x	60.853 (70)
Fipronil (5000 ppm + 5.26 g soil particles)	160	1.183 (1.115-1.256)	y = -0.4596.274x	14.354 (70)
Fipronil (500 ppm+ 0 g soil particles)	160	4.030 (3.8319-4.238)	y = -2.7754.584x	65.484 (70)
Fipronil (500 ppm + 4.44 g soil particles)	160	2.427 (2.300-2.561)	y = -2.102 + 5.460x	50.426 (70)

Discussion

Our study showed that (i) surface treatment of nine insecticides had concentration-dependent toxicity against *S. invicta* workers; (ii) beta-cypermethrin (0.5, 5, 50, 500, or 5000 ppm) and rotenone (0.05, 5, 50, or 5000 ppm) significantly decreased the number of foraging ants in the laboratory, and beta-cypermethrin (500 or 5000 ppm), fipronil (500 or 5000 ppm), chlorfenapyr (5000 ppm), and rotenone (5000 ppm) significantly decreased the number of foraging ants in the field; (iii) fipronil, rotenone, and avermectin significantly induced particle-covering behaviors of *S. invicta* workers under both laboratory and field conditions; and (iv) using particles to artificially cover the surfaces previously treated with fipronil or rotenone (500 or 5000 ppm) significantly decreased the toxicity of these insecticides against *S. invicta* workers (with significantly higher LT₅₀ values).

Jr. Stringer et al. (1964) defined the slow acting insecticide as killing < 15% *S. invicta* workers at one day and > 89% ants at the end of the test period (14 day). On the contrary, a fast acting insecticide caused rapid mortality of ants in a short period of time. The fast- or slow-action of insecticides is concentration-dependent. For example, beta-cypermethrin (≥ 0.5 ppm), thiamethoxam (≥ 50 ppm), fipronil (≥ 50 ppm), indoxacarb (500 and 5000 ppm), chlorfenapyr (500 and 5000 ppm), and rotenone (500 and 5000 ppm) caused > 80% mortality of ants within 24 h, but the remained concentrations showed slower speeds of kill. For the two insecticides that can be used as bait toxicants, indoxacarb caused high mortality of *S. invicta* workers through contacting, whereas avermectin may be not quite effective as a contact-based insecticide for *S. invicta* control.

Few studies evaluated the repellency of insecticides against *S. invicta*. Wiltz et al. (2010) reported that significantly lower proportions of *S. invicta* workers passed the bridges treated with bifenthrin to reach a food source than that passed the bridges treated with water. This result was more likely due to the rapid lethal effect of bifenthrin, which reduced the level of recruitment, rather than the repellent effect of the insecticide (Wiltz et al. 2010). Likewise, we observed some dead ants on or around the squares treated with beta-cypermethrin, which may disturb the recruitment of ants. Our study also showed that rotenone decreased the number of foraging ants under laboratory and field conditions. In addition, the reduction in number of foraging ants caused by the high concentrations of fipronil and chlorfenapyr was evident in the field, though neither reduced the number of foraging ants in the laboratory. It worth noting that rotenone (except at the contraindications of 500 and 5000 ppm) and fipronil had slower action than beta-cypermethrin (Fig. 2), and few dead ants were observed on or around the squares treated with these insecticides. Therefore, the decrease of foraging ants may be due to the “true” repellent effect of rotenone and fipronil, instead of the reducing recruitment caused by rapid lethal effects of these insecticides.

The repellency of insecticides can be two-fold for fire ant control. On one hand, ants may avoid walking on the surfaces treated with a repelling insecticide, and therefore they would not acquire the lethal dose of insecticide. On the other hand, insecticide with strong repellency may prevent *S. invicta* from entering the treated area (Rust and Su 2010). Under both laboratory and field conditions, few food items placed on the squares treated with beta-cypermethrin (500 and 5000 ppm) and fipronil (5000 ppm) were transported by *S. invicta* at 24 h. These results showed that the repellency of beta-cypermethrin and fipronil may last longer than other insecticides. In a recent study aiming to screen effective fire ant repellents, only N, N-diethyl-*m*-toluamide (DEET) and eugenol can completely repel *S. invicta* foragers for 24 h in the field (Wen et al. 2020a). The present study showed that the repellent duration of beta-cypermethrin and fipronil may be comparable to DEET and eugenol. Therefore, area or periphery treatment of high concentrations of beta-cypermethrin and fipronil may effectively stop ants from entering or passing the treated area, and therefore protect people from fire ant stings.

In our study, significantly higher levels of particle-covering behavior were triggered by the high concentrations of fipronil (50, 500, and 5000 ppm), rotenone (5000 ppm), and avermectin (5000 ppm) in the laboratory. These results indicate that the particle-covering behavior is concentration-dependent. In accordance with laboratory results, *S. invicta* workers also covered the squares treated with high concentrations of fipronil, rotenone, or avermectin in the field. Similar behavior was observed when *S. invicta* workers use particles to cover the surfaces treated with fire ant repellents (Wen et al. 2016, 2020a, b). Wen et al. (2016) reported that *S. invicta* workers can access the squares smeared with essential balm by covering the treated zones with particles, but they failed to do so when no particles were available, indicating that particle-covering is essential for *S. invicta* workers to walk and search for food on the repellent surfaces. In the present study, high concentrations of rotenone (in the laboratory and field) and fipronil (in the field) repelled foraging ants and induced the relocation of particles. However, the particle-covering behavior of *S. invicta* workers may not be tightly associated with the repellency of pesticides because no significant amount of particles were found on surfaces treated with beta-cypermethrin, which showed strong repellency against *S. invicta* workers. On the contrary, *S. invicta* workers relocated significantly more particles on the squares treated with the high concentration of avermectin than the control squares, but this insecticide did not significantly reduce the number of foraging ants.

The insecticide resistance of pests has received great attention (e.g., Hemingway et al. 2000; Naqqash et al. 2016; Zhu et al. 2016). There were more than 11,000 separate records of resistance to 331 insecticidal compounds before 2016 (Khodaverdi et al. 2016). The insecticide resistance can be divided into physiological and behavioral resistance; however, study on fire ants has focused more on the physiological resistance. For example, the gene expression and o-demethylase activity of cytochrome P450 can be significantly induced in *S. invicta* workers exposed to fipronil (Zhang et al. 2016 a, b). Also, Xiong et al. (2019) reported that cytochrome P450 of *S. invicta* played an important role in detoxifying fipronil by transforming it into fipronil-sulfone. Compared to physiological resistance with complex biochemical and genetic mechanisms, behavioral resistance usually has been considered as “simply aversion behaviors either learned or based on simple repellency or avoidance” (Zalucki and Furlong 2017). Our study showed that *S. invicta* workers not only avoid accessing surfaces treated with some pesticides, but also modify the treated surfaces with particles to reduce the toxicity of certain insecticides (e.g., rotenone and fipronil). Some eusocial insects such as ants and termites usually bury or cover the corpses with particles (e.g., Hölldobler and Wilson 1990; Sun and Zhou 2013; Wang et al. 2013). This strategy can effectively isolate microbial pathogens within corpses, and therefore prevent disease transmission (Wang et al. 2013). As far as we know, our study provides the first evidence that *S. invicta* workers also used particles to keep themselves off the insecticides, which can be considered as a unique behavioral resistance strategy in ants. We suggest to include this behavior in evaluating the effectiveness of insecticides for fire ant control in future studies.

Declarations

Author Contribution

ChW, JC, YF, ZW, LW, XW, TM, and CW conceived and designed the experiments. ChW, LS, JZ, and YH conducted experiments. ChW, LS, XC, JC, TM, and CW analyzed data. ChW, JC, TM, and CW wrote the manuscript. All authors read and approved the manuscript.

Acknowledgments

We thank Dr. Hamed Abbas (USDA-ARS, Stoneville, MS, USA) for reviewing an earlier version of this manuscript. This work was supported by the Pearl River S&T Nova Program of Guangzhou (201806010182).

Conflict of interest The authors declare that they have no conflict of interest.

References

1. Ascunce MS, Yang CC, Oakey J, Calcaterra L, Wu WJ, Shih CJ, Goudet J, Ross KG, Shoemaker1 DW (2011) Global invasion history of the fire ant *Solenopsis invicta*. *Science* 331:1066–1068. <https://doi.org/10.1126/science.1198734>
2. Chen HY, Feng X, Lu LH, Liu J, Zhou XM, Mo Y (2006) Bioassay of contact insecticides to red imported fire ant, *Solenopsis invicta* Buren. *Guangdong Agricultural Sciences* 5:28–30 (in Chinese)
3. Chen J, Oi DH (2020) Naturally occurring compounds/materials as alternatives to synthetic chemical insecticides for use in fire ant management. *Insects* 11:758. <https://doi.org/10.3390/insects11110758>
4. Cole LM, Nicholson RA, Casida JE (1993) Action of phenylpyrazole insecticides at the GABA-gated chloride channel. *Pestic Biochem Physiol* 46:47–54. <https://doi.org/10.1006/pest.1993.1035>
5. Field LM, Davies TE, O’Reilly AO, Williamson MS, Wallace BA (2017) Voltage-gated sodium channels as targets for pyrethroid insecticides. *Eur Biophys J* 46:675–679. <https://doi.org/10.1007/s00249-016-1195-1>
6. Hemingway J, Field L, Vontas J (2002) An overview of insecticide resistance. *Science* 298:96–97. <https://doi.org/10.1126/science.1078052>

7. Hölldobler B, Wilson EO (1990) The ants. Belknap Press of Harvard University, Cambridge
8. Hosie AM, Baylis HA, Buckingham SD, Sattelle DB (1995) Actions of the insecticide fipronil, on dieldrin-sensitive and -resistant GABA receptors of *Drosophila melanogaster*. Brit J Pharmacol 115:909–912. <https://doi.org/10.1111/j.1476-5381.1995.tb15896.x>
9. IRAC (2019) Mode of action classification scheme. Insecticide resistance action committee (Version 9.0)
10. Jr Stringer CE, Lofgren CS, Bartlett FJ (1964) Imported fire ant toxic bait studies: Evaluation of toxicants. J Econ Entomol 57:941–945. <https://doi.org/10.1093/jee/57.6.941>
11. Junji S, Tatsuya I, Yasuo K, Yoshiaki I, Chiharu M, Yokichi T et al (2002) Synthesis and activity of novel acaricidal/insecticidal 2, 4-diphenyl-1, 3-oxazolines. J Pestic Sci 27:1–8. <https://doi.org/10.1584/jpestics.27.1>
12. Khodaverdi H, Fowles T, Bick E, Nansen C (2016) Does drought increase the risk of insects developing behavioral resistance to systemic insecticides? J Econ Entomol 109:2027–2031. <https://doi.org/10.1093/jee/tow188>
13. Li FG, Ai GM, Li YS, Zhu CY, Gao XW (2013) Progress on mechanism of action and insecticide resistance of the oxadiazine indoxacarb in insects. Agrochemicals 52:558–560 (in Chinese)
14. Mao LX, Henderson G, Scherer CW (2011) Toxicity of seven termiticides on the formosan and eastern subterranean termites. J Econ Entomol 104:1002–1008. <https://doi.org/10.1603/EC11005>
15. Naqqash MN, Gökçe A, Bakhsh A, Salim M (2016) Insecticide resistance and its molecular basis in urban insect pests. Parasitol Res 115:1363–1373. <https://doi.org/10.1007/s00436-015-4898-9>
16. Ning D, Yang F, Xiao Q, Ran H, Xu Y (2019) A simple and efficient method for preventing ant escape (hymenoptera: formicidae). Myrmecol News 29:57–65. https://doi.org/10.25849/myrmecol.news_029:057
17. Noguchi K, Yulia GR, Edgar B, Frank K (2012) nparLD: An R software package for the nonparametric analysis of longitudinal data in factorial experiments. J Stat Softw 12:1–23. <https://doi.org/10.18637/jss.v050.i12>
18. Oi DH, Oi FM (2006) Speed of efficacy and delayed toxicity characteristics of fast-acting fire ant (Hymenoptera: Formicidae) baits. J Econ Entomol 99:1739–1748. <https://doi.org/10.1603/0022-0493-99.5.1739>
19. Qin W, Chen X, Hooper-Bùi LM, Cai J, Wang L, Sun Z, Wen X, Wang C (2019) Food-burying behavior in red imported fire ants (Hymenoptera: Formicidae). PeerJ 7:e6349. <https://doi.org/10.7717/peerj.6349>
20. Rust MK, Su NY (2012) Managing social insects of urban importance. Annu Rev Entomol 57:355–375. <https://doi.org/10.1146/annurev-ento-120710-100634>
21. Sun Q, Zhou X (2013) Corpse management in social insects. Int J Biol Sci 9:313–321. <https://doi.org/10.7150/ijbs.5781>
22. Vais H, Williamson MS, Devonshire AL, Usherwood PN (2010) The molecular interactions of pyrethroid insecticides with insect and mammalian sodium channels. Pest Manag Sci 57:877–888. <https://doi.org/10.1002/ps.392>
23. Vinson SB (2013) Impact of the invasion of the imported fire ant. Insect Sci 20:439–455. <https://doi.org/10.1111/j.1744-7917.2012.01572.x>
24. Wang C, Hendeson G, Gautam BK (2013) Lufenuron suppresses the resistance of Formosan subterranean termites (Isoptera: Rhinotermitidae) to entomopathogenic bacteria. J Econ Entomol 106:1812–1818. <https://doi.org/10.1603/EC13068>
25. Wang L, Zeng L, Xu Y, Lu Y (2020) Prevalence and management of *Solenopsis invicta* in China. NeoBiota 54:89–124. <https://doi.org/doi:10.3897/neobiota.54.38584>
26. Wang L, Zhao F, Tao Q, Li J, Xu Y, Li Z, Lu Y (2020) Toxicity and sublethal effect of triflumezopyrim against red imported fire Ant (Hymenoptera: Formicidae). J Econ Entomol 113:1753–1760. <https://doi.org/10.1093/jee/toaa083>
27. Wen C, Chen J, He Y, Wang F, Qian C, Wen J, Wen X, Wang C (2020a) Electrophysiological and behavioral responses of red imported fire ants (Hymenoptera: Formicidae) to an essential balm and its components. Pest Manag Sci 77:1971–1980. <https://doi.org/10.1002/ps.6225>
28. Wen C, Chen J, Qin WQ, Chen X, Cai J, Wen J, Wen X, Wang C (2020b) Red imported fire ants (Hymenoptera: Formicidae) covered inaccessible surfaces with particles to facilitate food search and transportation. Insect Sci. <https://doi.org/10.1111/1744-7917.12891>
29. Wen Y, Ma T, Chen X, Liu Z, Zhu C, Zhang Y, Strecker R, Henderson G, Hooper-Bùi LM, Chen X, Sun Z, Wen X, Wang C (2016) Essential balm: A strong repellent against foraging and defending red imported fire ants (Hymenoptera: Formicidae). J Econ Entomol 109:1827–1833. <https://doi.org/10.1093/jee/tow130>
30. Williams DF (1985) Laboratory and field evaluation of avermectin against the imported fire ant. Southwest Entomol 7:27–33
31. Wiltz BA, Suiter DR, Gardner WA (2010) Activity of bifenthrin, chlorfenapyr, fipronil, and thiamethoxam against red imported fire ants (Hymenoptera: Formicidae). J Econ Entomol 103:754–761. <https://doi.org/10.1603/ec09350>
32. Xiong T, Qiu XH, Ling SQ, Liu JL, Zeng XN (2019) Interaction of fipronil and the red imported fire ant (*Solenopsis invicta*): Toxicity differences and detoxification responses. J Insect Physiol 115:20–26. <https://doi.org/10.1016/j.jinsphys.2019.03.001>
33. Zalucki MP, Furlong MJ (2017) Behavior as a mechanism of insecticide resistance: evaluation of the evidence. Curr Opin Insect Sci 21:19–25. <https://doi.org/10.1016/j.cois.2017.05.006>
34. Zhang B, Kong F, Wang H, Gao X, Zeng X, Shi X (2016) Insecticide induction of O-demethylase activity and expression of cytochrome P450 genes in the red imported fire ant (*Solenopsis invicta* Buren). J Integr Agr 15:135–144 (in Chinese)
35. Zhang B, Lei Z, Cui R, Zeng X, Gao X (2016) Cloning and expression of multiple cytochrome p450 genes: induction by fipronil in workers of the red imported fire ant (*Solenopsis invicta* Buren). Plos One 11:e0150915. <https://doi.org/10.1371/journal.pone.0150915>

36. Zhang LL (2014) The stress effects of spinetoram on *Helicoverpa armigera* and its influence on natural enemies. Dissertation. *China Agricultural University*.
37. Zhu F, Lavine L, O'Neal S, Lavine M, Foss C, Walsh D (2016) Insecticide resistance and management strategies in urban ecosystems. *Insects* 7:2. <https://doi.org/10.3390/insects7010002>

Figures

Figure 1

Bioassays to investigate behaviors of *Solenopsis invicta* workers in response to squares smeared with insecticides under laboratory (A) or field (B) conditions.

Figure 2

Mortality of *Solenopsis invicta* workers exposed to the surface treated with different concentrations of beta-cypermethrin (A), thiamethoxam (B), fipronil (C), indoxacarb (D), chlorfenapyr (E), rotenone (F), spinetoram (G), avermectin (H), or chlorantraniliprole (I).

Figure 3

Average number of foraging ants on the square treated with different concentrations of beta-cypermethrin (A), thiamethoxam (B), fipronil (C), indoxacarb (D), chlorfenapyr (E), rotenone (F), spinetoram (G), avermectin (H), or chlorantraniliprole (I) under laboratory conditions. Boxes show the 25th percentile, 50th percentile (median), 75th percentile, and whiskers show the maximum and minimum value of the data. Different letters indicate significant differences ($P < 0.05$).

Figure 4

Number of food items that were not transported by ants when placed on the square treated with different concentrations of beta-cypermethrin (A), thiamethoxam (B), fipronil (C), indoxacarb (D), chlorfenapyr (E), rotenone (F), spinetoram (G), avermectin (H), or chlorantraniliprole (I) under laboratory conditions. Note that few food items on the squares smeared with beta-cypermethrin (500 or 5000 ppm), thiamethoxam (5000 ppm), or fipronil (5000 ppm) were transported at the end of the experiment (24 h).

Figure 5

Dry weight of particles relocated onto the square treated with different concentrations of beta-cypermethrin (A), thiamethoxam (B), fipronil (C), indoxacarb (D), chlorfenapyr (E), rotenone (F), spinetoram (G), avermectin (H), or chlorantraniliprole (I) under laboratory conditions. Boxes show the 25th percentile, 50th percentile (median), 75th percentile, and whiskers show the maximum and minimum value of the data. Different letters indicate significant differences ($P < 0.05$).

Figure 6

Particle-covered areas on squares treated with different concentrations of beta-cypermethrin (A), thiamethoxam (B), fipronil (C), indoxacarb (D), chlorfenapyr (E), rotenone (F), spinetoram (G), avermectin (H), or chlorantraniliprole (I) under laboratory conditions. Boxes show the 25th percentile, 50th percentile (median), 75th percentile, and whiskers show the maximum and minimum value of the data. Different letters indicate significant differences (P < 0.05).

Figure 7

Particle weight and covered areas on the square treated with 500- or 5000-ppm solutions of beta-cypermethrin, thiamethoxam, fipronil, indoxacarb, chlorfenapyr, rotenone, spinetoram, avermectin, chlorantraniliprole, or distilled water (controls) under field conditions. Boxes show the 25th percentile, 50th percentile (median), 75th percentile, and whiskers show the maximum and minimum value of the data. Different letters indicate significant differences ($P < 0.05$).

Figure 8

Mortality of *Solenopsis invicta* workers exposed to surfaces treated with rotenone (A) or fipronil (B) that were either artificially covered with soil particles or not. Different letters indicate significant differences within each time period ($P < 0.05$).

Supplementary Files

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