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ORIGINAL RESEARCH PAPER

Acute toxicity of typical ant control agents to the red imported fire ant, *Solenopsis invicta* (Hymenoptera: Formicidae)

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Abstract
The red imported fire ant *Solenopsis invicta* Buren (Hymenoptera: Formicidae), is a serious invasive alien ant around the world and has expanded its invasive range to the Pacific Rim since the early 2000s. It was first reported in Japan in 2017, and its entry through cargo has been reported numerous times in many ports. Colonies have been found in Tokyo Port since 2019, and now it is an urgent issue to prevent further invasion and establishment. Chemical control is the best tested method of insect control, but we have little information on the efficacy of insecticides against *S. invicta* in Japan. Here, we conducted acute toxicity assays of six quick-acting pyrethroids (transfluthrin, prallethrin, phenothrin, permethrin, metofluthrin, and pyrethrin) for killing adults and five new-type insecticides (fipronil, thiamethoxam, indoxacarb, imidacloprid, and hydramethylnon) for controlling colonies with toxic baits. We found that the LD_{50} from six pyrethroids were comparable to each other. The ED_{50} causing abnormal behaviors were smaller than LD_{50}, but some ants recovered from paralysis within 12 h. Fipronil showed the lowest LD_{50} suggesting this chemical is the most promising agent for controlling *S. invicta*. Our results promise to develop a method for the chemical control of *S. invicta*.

Keywords Invasive alien ants · Chemical control · Fipronil · *Solenopsis* · Synthetic pyrethroids

Introduction

The harm caused by invasive alien species to native ecosystems and biodiversity is becoming more serious (Bellard et al. 2016; Blackburn et al. 2019; Clavero and García-Berthou, 2005). In particular, invasive alien ants have proliferated explosively in their invasive range, causing serious damage not only to native ecosystems, but also to human life (Holway et al. 2002; Suarez et al. 2010). In fact, on the IUCN list of the 100 most invasive alien species, the group with the largest number of species is the Formicidae (Lowe et al. 2000). Human activity has been introducing invasive alien ants worldwide (Suarez et al. 2010). Asia has been invaded by ants of diverse origin and taxonomy, beginning with the tropical fire ant, *Solenopsis geminata* (Fabricius) (Hymenoptera: Formicidae) (Gotzek et al. 2015), followed by the Argentine ant *Linepithema humile* (Mayr) (Hymenoptera: Formicidae), native to South America, in the 1990s (Sugiyama 2000) and, recently, the browsing ant, *Lepisiota frauenfeldi* (Mayr) (Hymenoptera: Formicidae), native to southern Europe (Ministry of the Environment 2019).

The most serious invasive alien ant species is the red imported fire ant *S. invicta* Buren (Hymenoptera: Formicidae) (Tschinkel 2006; Wetterer 2013; Wylie et al. 2020). This species originated in South America and invaded the USA in the 1930s (Wetterer 2013). There, it causes USD 6 billion worth of damage each year over a wide range, harming human health, agriculture, and electrical equipment, among others (Drees and Lard 2006; Gutrich et al. 2007). Its invasion from the USA into the Pacific Rim began with Australia in 2001 and continued with New Zealand and Taiwan in 2004 and China in 2005 (Wylie et al., 2020). It has become established in all of these countries, expect New Zealand, where early eradication was successful. Since 2017, furthermore, there have been continual reports of unintentional introductions of *S. invicta* into South Korea and Japan (Lyu and Lee 2017; Ujiyama and Tsuji 2018). Remarkably, from October 2019, *S. invicta* colonies with a lot of reproductives were continuously discovered at Tokyo

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Port (Ministry of the Environment 2020a). Furthermore, in September 2020, a mature nest with 50 newly-emerged queens was found at Nagoya Port (Ministry of the Environment 2020b). In this imminent situation, there is an urgent need to establish a method for the control of *S. invicta*.

A strategy for the control of *S. invicta* should follow three stages. The first stage is pre-invasion control: that is, preventing *S. invicta* from being transported to Japan. The second stage is pre-establishment control: that is eradication of *S. invicta* in cargo. The third stage is post-establishment control: that is, eradication of wild nests already established. Of these stages, pre-invasion control is the most effective (Rabitsch 2011), but its implementation is problematic because it requires the cooperation of exporting countries. Therefore, we need urgently to establish methods of pre- and post-establishment control. Past successful cases of the eradication of invasive alien ants show that insecticides use, i.e., chemical control, is the most promising method (Hoffmann et al. 2016).

In pre-establishment control, applying quick-acting but human-safe chemicals into sea cargos is necessary to ensure smooth trading. Pyrethroids are desirable insecticides for their quick-acting insect-specific features, because they quickly act on insect nerve axons to open sodium channels in the nerve membranes and induce overexcitement in insect (Palmquist et al. 2012). Indeed, spraying pyrethroids into a sea cargo and sealing the sea cargo for time has high lethality against invasive alien ants (Sasaki et al. 2019). To repel and keep *S. invicta* out of sea cargo, microencapsulated allyl isothiocyanate, a natural compound in wasabi (*Eutrema japonicum*), has proved effective (Hashimoto et al. 2019). In post-establishment control, toxic baits with slow-acting chemicals are effective (Hoffman et al. 2016). Quick-acting insecticides may kill workers but not queens because the reproductive caste (queen) of a colony is strongly protected from direct chemical contact in the social insects. Toxic baits with slow-acting chemicals can be brought back to the nest by workers and shared with the queens living deep inside the nest though. In Japan, fipronil bait, which is used to control the Argentine ant (Inoue et al. 2015; Sakamoto et al. 2019), is now used for the emergency control of *S. invicta* colonies. Fipronil has strong insecticidal properties by inhibiting the γ-aminobutyric acid (GABA) receptor and disrupting the neurotransmitter action of insects (Wang et al. 2016).

Nevertheless, it is necessary to examine which insecticide is the most effective against *S. invicta*, because other insecticides which have different mechanisms of action—namely two neonicotinoids (thiamethoxam, imidacloprid), an oxadiazine (indoxacarb), and an amidinohydrazone (hydramethylnon)—are also used to control invasive alien ants (Blight et al. 2011; Boser et al. 2017; Hoffmann et al. 2016; Sarty 2007). Neonicotinoid insecticides act as agonists at the nicotinic acetylcholine receptors (nAChRs) and cause nerve paralysis (Tomizawa and Casida 2005). Oxadiazine insecticides block insect sodium channels in nerve preparations and isolated neurons (Song et al. 2006). And amidinohydrazone insecticides work by inhibiting the mitochondrial electron transfer system complex III and cause death through inhibition of cellular respiration (Hooper-Bui et al. 2015). Selection of the most suitable agent is key to successful control (Hoffman et al. 2016). Although some studies have reported the acute toxicity of fipronil to *S. invicta* (Xiong et al. 2019) and of permethrin to the fire ants *S. saevissima* (Smith) (Hymenoptera: Formicidae) (Moreno et al. 2009, 2017), few studies have compared the effectiveness of multiple insecticides against *S. invicta*. Seagraves and McPherson (2003), for example, compared efficacy among four commonly used insecticides (Methomyl, chlorpyrifos, acephate, and lambda-cyhalothrin) throughout the USA. Since those chemicals are outdated for invasive alien ant control, we compared the efficacy of the above-mentioned insecticides for current invasive alien ant control.

Here, we estimated the relative efficacies of various insecticides in an acute toxicity assay against *S. invicta* worker ants. The methods for acute toxicity assay include both topical and oral exposure. Oral exposure can accurately quantify the amount of insecticide ingested at the individual level, but this method is particularly difficult for small-sized insects like ants. Since some of the previous studies show strong correlations between topical and oral toxicity in the hymenopteran insects (Sanchez-Bayo and Goka 2014), we chose the topical exposure in this study. The basic toxicity data obtained will be very useful for optimizing pre- and post-establishment control of *S. invicta*.

Materials and methods

Ants

Two thousand live worker ants of *S. invicta* were collected from an invasion site in Tucheng District, New Taipei City, Taiwan (23,624°58′41.1″ N, 121°26′49.6″ E) and held in aluminum boxes (16 cm × 11 cm × 4 cm) until the toxicity test. Each box held a 15-mL distillation centrifuge tube filled with tap water and plugged with cotton as a water supply, and an insect-pet food jelly (Fujikon, Osaka, Japan) as a food resource. The jelly was changed every day. The ants were held in a rearing room before and during the experiments (25 °C; 70% RH) in Taipei City.

Insecticides

The experiments used 11 insecticides: six quick-acting synthetic pyrethroids (transfluthrin, prallethrin, phenothrin, permethrin, metofluthrin, and pyrethrin), and five new-type insecticides (fipronil, thiamethoxam, indoxacarb, imidacloprid, and hydramethylnon) (Table 1). Each insecticide was purchased from Sigma-Aldrich (St. Louis, MO, USA). Each insecticide was made up as 100 ppm stock solution in acetone, from which further concentrations were prepared by serial dilution to 10, 1.0, 0.5, and 0.1 ppm. Acetone only was used as a control.

Acute toxicity test

Just before the experiment, the ants were anesthetized by placing their rearing box in a large aluminum container (20 cm × 16 cm × 8 cm) filled with crushed ice. A hundred anesthetized ants per experiment were held in an aluminum foil dish (8 cm in diameter × 3 cm deep) placed in a crashed-ice-filled container until acute toxicity tests were performed, using 10 ants per concentration. To avoid oral exposure of insecticide via nestmate grooming, the acute toxicity test was conducted individually. Anesthetized ants were placed on a filter paper with featherweight forceps (Bioquip, Compton, CA, USA), then, the test doses of insecticides were delivered in 1 µl acetone to the dorsal thorax of each ant through a 10-µL microsyringe (SGE, Malborne, Vic., Australia). The treated ants were dropped individually into a 120-mL clear plastic rearing cup lined with a 5.5-cm-diameter filter paper. A moistened melamine sponge (1 cm × 1 cm × 1 cm) was placed in the cup for hydration and the lid was kept closed during the experiment. To confirm the effectiveness of the insecticide, we observed the condition of the ants examined. The condition of each ant was observed for different length of time depending on the quick-acting and slow-acting insecticides. The first observation for each treatment was made right after the insecticide applications for all ants under the same treatment (that took 10 min to complete), followed by every 12-h observations up to 24 h for the six pyrethroids and up to 72 h for the five new-type insecticides. At the same time, 10 anesthetized ants were observed as controls of any negative effect of anesthesia. The ants used for the acute toxicity assay in this study were randomly selected from worker ants in all size (2–6 mm), because our preliminary experiments showed no clear difference in insecticidal sensitivity by size (data not shown). The experimental design (concentrations of insecticide and the number of observation days) of this study was based on a preliminary experiment showing sufficient effects on two native ant species *Formica japonica* Motschoulsky (Hymenoptera: Formicidae) and *Tetramorium tsushimae* Emery (Hymenoptera: Formicidae) in Japan.

Table 1 Insecticides used in experiments

Category	Insecticide	Purity (%)	IRAC codes
Pyrethroid	Transfluthrin	98.5	3A
Pyrethroid	Prallethrin	93.4	3A
Pyrethroid	Phenothrin	96.9	3A
Pyrethroid	Permethrin	91.0	3A
Pyrethroid	Metofluthrin	96.4	3A
Pyrethroid	Pyrethrin	46.0*	3A
Phenylpyrazole	Fipronil	99.4	2B
Neonicotinoid	Thiamethoxam	99.0	4A
Neonicotinoid	Imidacloprid	99.6	4A
Oxidiazine	Indoxacarb	98.0	22A
Aminohydrazone	Hydramethylnon	98.0	20A

*Natural extract from flowers of pyrethrum (*Tanacetum cinerariifolium*)

Data analysis

During the observation of the treated individuals, we observed ants with normal behavior, ants that died, and other ants with abnormal behavior. Thus, we defined mild behavioral abnormalities as "inhibited" and severe behavioral abnormalities as "immobile" based on the following characteristics. For accurate measurement, by lightly tapping the cup wall, we determined the condition of each ant:

1. normal__capable of moving with no sign of uncoordinated or uncontrolled activities.
2. Inhibited__capable of moving but with uncontrolled leg movements.
3. Immobile__incapable of any movement but still responding to stimuli.
4. Dead__showing no movement and no response.

Based on the mortality rates for each concentration of the insecticide, the lethal dose of each insecticides to 50% of the population (LD_{50}) was calculated by probit analysis in R (ver. 3.4.2.) software. The median effective dose (ED_{50}) that caused either “inhibited” or “immobile” (used as endpoints) was calculated in the same way.

Results

Evaluation of six pyrethroids

Pyrethrin had clearly higher LD_{50} values than the others at 12 and 24 h (Fig. 1). There were little differences in LD_{50} values between 12 and 24 h in any pyrethroids. These results indicate the very quick lethal effect of each pyrethroid. All ants were rendered immobile immediately after treatment with all pyrethroids at ≥ 10 ng/ant, and most died, although some recovered (Fig. S1). No pyrethroid inhibited movement (Fig. S1). In contrast, ED_{50} increased from first observation (within 10 min) to 12 or 24 h with every pyrethroid

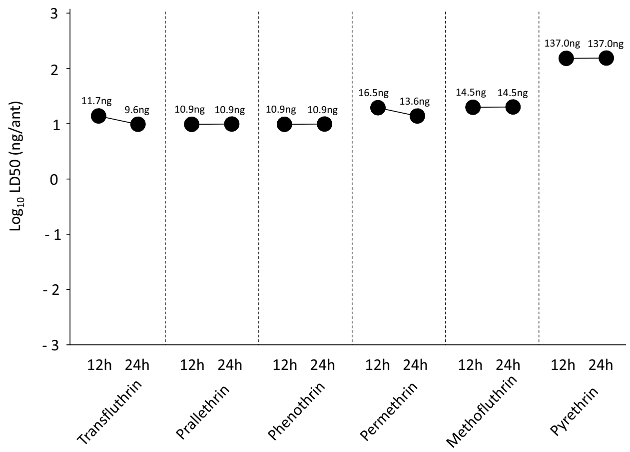

Fig. 1 LD_{50} values of six insecticide candidates for pre-establishment control of *S. invicta*. Numbers above symbols indicate actual LD_{50} values

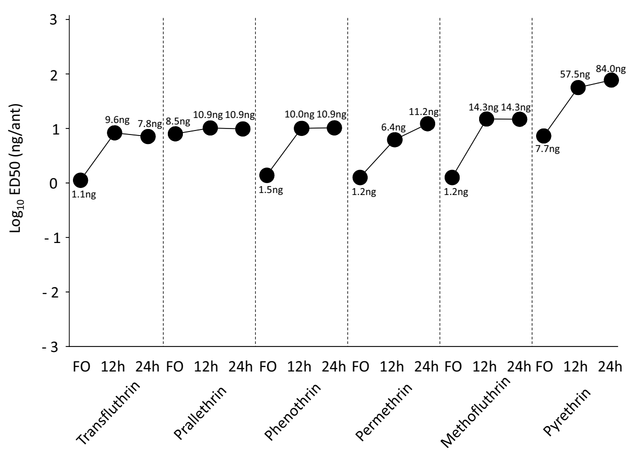

Fig. 2 Immobility ED_{50} values of six insecticide candidates for pre-establishment control of *S. invicta*. Numbers above symbols indicate actual ED_{50} values. First observation (FO) was conducted within 10 min after topical assay

examined (Fig. 2). ED_{50} values were smaller than LD_{50} values. The cold anesthesia and acetone control had no negative effect ($N = 60$ each).

Evaluation of five new-type insecticides

Mortalities caused by the new-type insecticide tended to increase up to 72 h (Table 2). In particular, both fipronil and thiamethoxam had strong effects. The LD_{50} values of fipronil was one to two orders of magnitude smaller than those of the other insecticides (Fig. 3). Thiamethoxam was the next effective insecticide, whereas indoxacarb and imidacloprid were inferior. The LD_{50} values of hydramethylnon could not be estimated, because there were no fatalities even at the highest concentrations. No ants showed inhibition of movement or immobility at first observation (Fig. S2). At all concentrations of indoxacarb, no ants showed inhibition or immobility until 24 h (100 ng/ant only). Only imidacloprid resulted in recovery of ED_{50} values between 48 and 72 h (Figs. 4, 5). The ED_{50} values of most insecticides for immobility (Fig. 4) were not clearly different from those for inhibition (Fig. 5), but the ED_{50} values of indoxacarb for immobility were smaller than those for inhibition. Notably, hydramethylnon did not cause inhibition or immobility. The cold anesthesia and acetone controls had no negative effect ($N = 50$ each).

Discussion

Insecticide candidates for pre-establishment control of *S. invicta*

Overall, there was little difference between the LD_{50} values of the six pyrethroids except for pyrethrin, which is an agent derived from flowers of pyrethrum (*Tanacetum cinerariifolium*), and all were highly effective at killing *S. invicta* worker ants (Fig. 1; Table 3). The increase in the ED_{50} values for immobility over time, i.e., increase in the number of ants that behave normally, indicates that the pyrethroids could cause temporary knockdown, from which the ants were capable of recovering within 12 h (Fig. 2). Following treatment with permethrin at 1–100 ng/ant, some ants had recovered by 24 h after immobility at 12 h (Fig. S1d). The knockdown effect of pyrethroids is caused by inhibition of the nervous system via overstimulation of sodium channels (Rehman et al. 2014). Pyrethroids have knockdown effects in insects in general, including major pests such as the common house mosquito *Culex pipiens* Linnaeus (Diptera: Culicidae), the housefly *Musca domestica* Linnaeus (Diptera: Schizophora), and the German cockroach *Blattella germanic*a Linnaeus (Blattaria: Blattellidae) (Matsuo 2019; Palmquist et al. 2012; Rehman et al. 2014). The knockdown effect is rapid, but sometimes the pyrethroids are decomposed by metabolic enzymes in the insects, allowing recovery from paralysis (Rehman et al. 2014). In actual pre-establishment control programs, such recovery will enable the *S. invicta* to spread farther. Therefore, if pyrethroids are used, the exposure concentration, dose, and duration must be great to kill all ants.

Insecticide candidates for post-establishment control of *S. invicta*

Both fipronil and thiamethoxam promise prolonged and strong efficacy for post-establishment control (Fig. 3), and their ED_{50} values did not increase over time (Figs. 4, 5).

Table 2 Hourly mortality after application of five insecticide candidates for post-establishment control of *Solenopsis invicta*

Insecticide	Dose (ng/ant)	Hourly mortality ($N=10$; each insecticide, $N=50$; acetone)*						
		First observation**	12 h	24 h	36 h	48 h	60 h	72 h
Fipronil	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.5	0.0	0.0	0.0	0.1	0.4	0.5	0.5
	1	0.0	0.2	0.3	0.7	0.9	0.9	0.9
	10	0.0	0.9	1.0	1.0	1.0	1.0	1.0
	100	0.0	1.0	1.0	1.0	1.0	1.0	1.0
Thiamethoxam	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.1
	1	0.0	0.0	0.1	0.1	0.1	0.1	0.1
	10	0.0	0.2	0.6	0.7	0.9	0.9	0.9
	100	0.0	0.9	1.0	1.0	1.0	1.0	1.0
Indoxacarb	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	100	0.0	0.9	0.9	0.9	0.9	0.9	0.9
Imidacloprid	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.5	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	100	0.0	0.7	0.7	0.8	0.8	0.8	0.8
Hydramethylnon	0.1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	0.5	0.0	0.0	0.0	0.1	0.1	0.2	0.2
	1	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	10	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	100	0.0	0.0	0.0	0.0	0.0	0.0	0.0
Acetone	–	0.0	0.0	0.0	0.0	0.0	0.0	0.0

*1.0 = 100% mortality

**within 10 min after topical assay

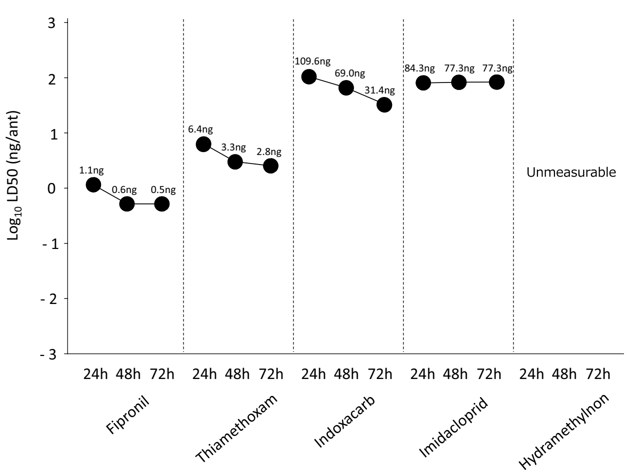

Fig. 3 LD_{50} values of five insecticide candidates for post-establishment control of *S. invicta*. Numbers above symbols indicate actual LD_{50} values

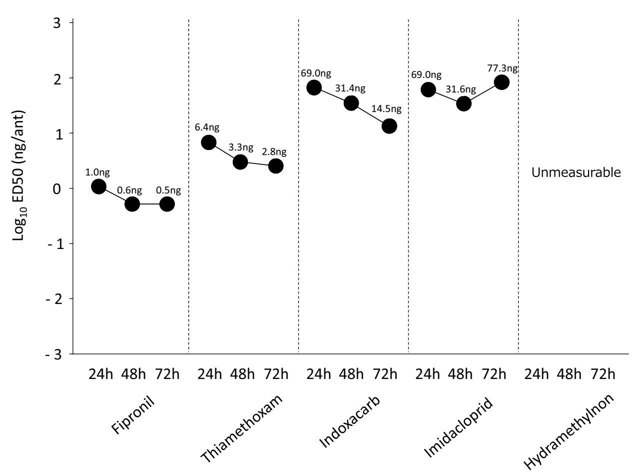

Fig. 4 Immobility ED_{50} values of five insecticide candidates for post-establishment control of *S. invicta*. Numbers above symbols indicate actual ED_{50} values

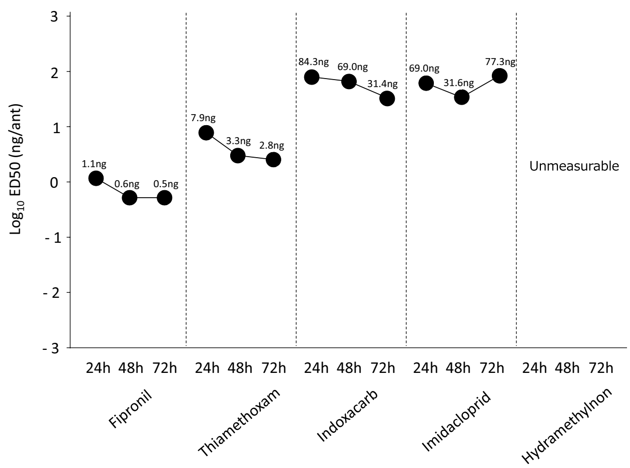

Fig. 5 Inhibition ED_{50} values of five insecticide candidates for post-establishment control of *S. invicta*. Numbers above symbols indicate actual ED_{50} values

These results indicate that the ants could not recover after exposure to these insecticides.

Notably, the LD_{50} values of fipronil were one–two orders of magnitude smaller than those of the other insecticides. They were close to those of previous studies using invasive populations of *S. invicta* in China (Xiong et al. 2019), so we expect the differences in sensitivity to fipronil among populations of *S. invicta* to be small.

These results indicate that fipronil is likely to be the most effective insecticide for chemical control of *S. invicta*. In fact, fipronil is already widely used for this. It has also been used to eradicate the Argentine ant in Japan (Inoue et al. 2015; Sakamoto et al. 2017, 2019).

We must always consider that insecticide use in the field may harm non-target invertebrates, including native ants (Hoffmann et al. 2016). However, since invasive alien ants generally dominate foraging behavior in invasion areas, it is likely that they will consume most of the poisonous bait laid (Human and Gordon 1996; Holway 1999). Therefore, chemical control using baits is likely to have a smaller impact on native surface-roaming organisms (Silverman and Brightwell 2008). In an eradication study using fipronil baits in Japan, native ant numbers recovered as Argentine ants declined at the initial stage of the eradication (Inoue et al. 2015). Then, continued baiting reduced the numbers and species of native ants and other arthropods, but they recovered after the chemical control ended with the eradication of the Argentine ants (Sakamoto et al. 2019). These results indicate that poisonous baits can harm native ants when the density of invasive alien ants is low, but that the native ants can recover after baiting ends. In other words, rapid control of non-native ants with appropriate chemicals will have less impact on the ecosystem in the long term. Therefore, quick eradication of *S. invicta* by chemical control would be appropriate for native ecosystem conservation. In addition, because the toxic bait contains both insecticides and attractants, selecting effective attractants for *S. invicta* (e.g., vegetable oil (Kafle et al. 2008)) is also a key to avoid killing non-target invertebrates.

Table 3 Hourly mortality after application of six insecticide candidates for pre-establishment control of *Solenopsis invicta*

Insecticide	Dose (ng/ant)	Hourly mortality ($N=10$; each insecticide, $N=60$; acetone)*		
		First observation**	12 h	24 h
Transfluthrin	0.1	0.0	0.0	0.0
	0.5	0.0	0.0	0.0
	1	0.0	0.1	0.1
	10	0.0	0.3	0.4
	100	0.0	1.0	1.0
Prallethrin	0.1	0.0	0.0	0.0
	0.5	0.0	0.0	0.0
	1	0.0	0.0	0.0
	10	0.0	0.4	0.4
	100	0.0	1.0	1.0
Phenothrin	0.1	0.0	0.0	0.0
	0.5	0.0	0.0	0.0
	1	0.0	0.0	0.0
	10	0.0	0.4	0.4
	100	0.0	1.0	1.0
Permethrin	0.1	0.0	0.0	0.0
	0.5	0.0	0.0	0.0
	1	0.0	0.0	0.0
	10	0.0	0.4	0.5
	100	0.0	0.9	0.9
Metofluthrin	0.1	0.0	0.0	0.0
	0.5	0.0	0.0	0.0
	1	0.0	0.0	0.1
	10	0.0	0.1	0.1
	100	0.0	1.0	1.0
Pyrethrin	0.1	0.0	0.0	0.0
	0.5	0.0	0.0	0.0
	1	0.0	0.0	0.0
	10	0.0	0.2	0.2
	100	0.0	0.4	0.4
Acetone	–	0.0	0.0	0.0

*1.0 = 100% mortality

**within 10 min after topical assay

Effectiveness of LD_{50} and ED_{50} values as measures of insecticide efficacy

In addition to the LD_{50} values to evaluate the efficacy, we also used two time-series ED_{50} values for behavioral abnormalities (inhibition and immobility) in *S. invicta* ants. The ED_{50} values confirmed that the ants recovered from the acute knockdown effects, especially of pyrethroids. They also revealed that the ants recovered from the effects of imidacloprid at between 48 and 72 h (Figs. 4, 5). In assessing the actual toxicity of insecticides, we need to establish the fatal dose to address concern that ants will show resilience. Therefore, in addition to the LD_{50} value, the ED_{50} value should be measured over time for both inhibition and immobility to ascertain the likelihood of recovery. Evaluation of efficacy after confirming the dose that leads to certain death should be the standard method of deciding insecticide.

Improvements in pre- and post-establishment control of *S. invicta*, and future issues

We used acute toxicity tests to assess the efficacy of 11 insecticides that are candidates for pre- and post-establishment control of *S. invicta*. For pre-establishment control, all pyrethroids except pyrethrin were equally effective. For post-establishment control in baits, fipronil gave the best control. On the contrary, because some insecticides are more effective by oral and intestinal absorption, and because contact with pesticides during larval stage is mostly limited through feeding, we are now conducting efficacy tests using active ant colonies (Sakamoto and Goka, in preparation). Insect growth regulator (IGR) insecticides such as pyriproxyfen and methoprene in baits have shown good effect in the post-establishment control of *S. invicta* colonies in Australia and New Zealand (Sarty 2007; Wylie et al. 2016) and may offer better control. The efficacy of these IGR insecticides cannot be assessed in acute toxicity tests using adult ants, so, we must assess it in colony-level test. Also, the acute toxicity of hydramethylnon in our experiments was lower than expected, at least by topical exposure. Since hydramethylnon is a slow-acting insecticide, we observed hydramethylnon-treated ants for an additional week and found no dead nor abnormal behaving individuals. It is necessary to clarify and compare the toxicity of other insecticides via colony experiments.

Supplementary Information The online version contains supplementary material available at https://doi.org/10.1007/s13355-021-00728-8.

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