J. Pestic. Sci. **47**(4), 208–212 (2022)
DOI: 10.1584/jpestics.D22-049

Journal of Pesticide Science

Technical Report

Acute toxicity of fipronil to an invasive ant, *Lepisiota frauenfeldi*

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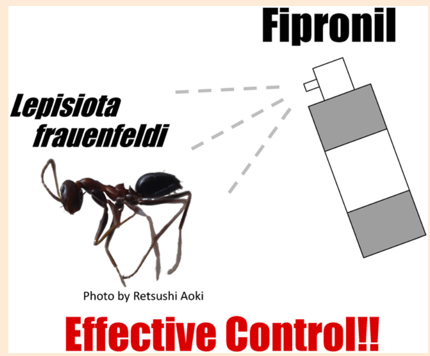
(Received August 15, 2022; Accepted October 10, 2022)

Slow-acting fipronil is one of the best components for controlling invasive ants. However, its efficacy against invasive *Lepisiota frauenfeldi*, which recently invaded Japan, remains unclear. Here, its acute toxicity to *Le. frauenfeldi* was assessed, and its lethal concentrations were compared with those against other invasive ants (*Linepithema humile* and *Solenopsis invicta*). The LC_{10} and LC_{50} values of fipronil for *Le. frauenfeldi* were significantly lower than the previously reported values for *Li. humile* and/or *S. invicta*, and its LC_{90} value against *Le. frauenfeldi* was in the same range as that required for *Li. humile* extermination. Additionally, *Le. frauenfeldi* can be more sensitive to fipronil than non-target arthropods. Therefore, recent fipronil-based *Li. humile* and *S. invicta* eradication/control programs may be effective against *Le. frauenfeldi* as well. Moreover, applying fipronil at dosages appropriate for *Le. frauenfeldi* would lead to effective *Le. frauenfeldi* extermination/control with low damage to other native species/ants.



Keywords: browsing ant, chemical control, eradication/extermination, invasive alien ants, slow-acting chemicals, toxic bait.

Introduction

The frequency of biological invasion events has been increased over the years due to increased international trade and tourism.[1,2] Among biological invaders, the number of species that are introduced unintentionally is proportional to the increased rate of importation of commodities.[3,4] In particular, invasive ants have serious impacts on native communities and ecosystems.[5,6] For example, *Linepithema humile* Mayr, 1868 (Argentine ant), *Solenopsis invicta* Buren, 1972 (red imported fire ant), and *Wasmannia auropunctata* Roger, 1863 (little fire ant) (Hymenoptera: Formicidae) are considered the world's most damaging invasive species[7]; thus, their eradication/control is proactively conducted in introduced areas.[8–10]

Chemical toxicity measures constitute the best tested methods for exterminating nuisance associated with pests, including ants,[11–13] even though they cause harm to non-target organisms.[14,15] Further, during the initial stage of invasion, when the distribution ranges of invasive species are narrow, such measures can lead to achievement of rapid eradication at a relatively low cost. Among the frequently used chemical toxicity measures, such as insecticidal spraying and fumigation, toxic baits with slow-acting chemicals, particularly fipronil, are among the best eradication/control agents against invasive ground-dwelling arthropods,[14,16,17] and have been recommended by the Japanese administrative organization for the control of invasive ants, including *Li. humile* and *S. invicta*.[18,19] However, it is thought that the quick-acting toxicants may only kill workers outside the nest; thus, are unlikely to lead to the collapse of the entire ant colony.[18]

Fipronil, a phenylpyrazole insecticide that disrupts neurotransmission in various insects via γ-aminobutyric acid (GABA) receptor inhibition,[20,21] is often preferentially used for exterminating invasive ants.[9,22] For example, Sakamoto and Goka[12] reported that fipronil has high insecticidal effects on *S. invicta* (48-hr LD_{50}: 0.6 ng/ant) at doses 2–3 orders of magnitude lower than those of other insecticides, such as pyrethroids and neonicotinoids.

Lepisiota frauenfeldi Mayr, 1855 (browsing ant) (Hymenop-

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Published online November 11, 2022

tera: Formicidae) is native to southern Europe, and has been unintentionally introduced in India, Southeast Asia, Guam, Australia, and Middle Eastern countries via the importation of commodities.[23)] For example, in Japan, it was first detected in Aichi Prefecture and Tokyo in 2017, and by 2022 it was detected in Osaka, Fukuoka, Kagoshima, and Okinawa Prefectures.[24,25)] Further, this species, which forms a super colony,[26)] mainly inhabits bare lands and developed areas, such as wharfs, airports, and urban spaces, and reportedly, it has negative ecological and agricultural impacts,[24)] and can diminish biodiversity in the introduced regions. In fact, in Australia, *Le. frauenfeldi* is a notorious and serious environmental pest, which eats, kills, and displaces not only native ant species, but also various other invertebrates in the infested area.[26,27)] Additionally, it can negatively impact agricultural and horticultural activities via "ant-Sternorrhyncha (particularly, scale insects which are one of the major agricultural pests) mutualisms".[26)] This mutualism has been found to exert a wide range of effects on arthropods, and it also eventually affects plant health.[28,29)] Based on previously reported findings, *Le. frauenfeldi* has been listed as an invasive alien species (IAS) under the Invasive Alien Species Act of Japan since 2020,[30)] and its eradication/control using chemical agents has been initiated in several areas, including Aichi and Okinawa prefectures. Although it is important to initiate a prompt response to eradicate invasive *Le. frauenfeldi*, it is unclear if the most effective control strategies (e.g., exposure concentration) against this ant have been fully explored.

Herein, the acute toxicity of fipronil to invasive *Le. frauenfeldi* was assessed under laboratory conditions, and the fipronil sensitivity of *Le. frauenfeldi* was compared with those of two other known invasive ants (*Li. humile* and *S. invicta*).[14,31)]

Materials and methods

1. *Test species*

According to Sunamura *et al.*,[13)] to ensure the effective and rapid eradication of *Le. frauenfeldi*, workers, which are indispensable for sustaining ant colony organization, should be primarily targeted. In this study, *Le. frauenfeldi* workers were sampled from the Tobishima wharf (30°02′03.7″N, 136°49′20.4″E), Nagoya City, Aichi Prefecture in October 2017, before their designation as IAS. The collected individuals were kept in plastic cases (300-mm length×155-mm width×155-mm depth) containing dry cotton filled with 25% sucrose solution for three days to allow for acclimation to the environment. This was then followed by the acute toxicity tests. It is also worth noting that the tested individuals were reared in an incubator (LH-30-8CT, Nippon Medical & Chemical Instruments Co., Ltd., Osaka) maintained at a constant temperature of 22±1°C.

2. *Oral acute toxicity bioassays of fipronil to* Lepisiota frauenfeldi *workers*

Oral acute toxicity bioassays of fipronil in *Le. frauenfeldi* workers were performed as previously described by Hayasaka *et al.*[14)] In brief, commercial fipronil [Prince Flowable, fipronil/water, and surfactant (5 : 95, v/v)] (BASF Japan Ltd., Tokyo), which was used in this study, was dissolved in dechlorinated tap water to prepare test solutions.

Thereafter, the individuals tested were fed via a melamine sponge (1 cm^3) soaked with either 0.5 mL of 25% sucrose water that was spiked with 0.5 mL of fipronil solution (treatment group) or with 1.0 mL of 25% sucrose water (control). Furthermore, in this study, the nominal concentration of fipronil varied in the ranged 98–6250 μg/L (no. for a fipronil concentration of 7), and the concentration ratio between successive solutions was 2.0. Further, the fipronil solutions were prepared via serial dilution of the stock solution using aerated tap water. Five *Le. frauenfeldi* individuals were placed on a melamine sponge filled with test solution at the center of a 90-mm petri dish, and each dish was sealed to prevent the escape of the specimens. Four replicates were used for each concentration (treatment). Mortality, which was defined as the absence of movement after the cover of the dish was gently flicked, was checked at 48 hr before the commencement of the experiments; this was to enable the calculation of different lethal concentrations (LC_{10}, LC_{50}, and LC_{90}). The survival rate of the test species in the control group throughout the experiments was 100% (20/20 individuals).

3. *Statistical analyses*

All statistical analyses were conducted using R software version 4.2.1.[32)] The lethal concentration (LC_{10}, LC_{50}, and LC_{90}) values of fipronil with respect to *Le. frauenfeldi* workers were calculated based on data from the 48-hr acute toxicity bioassays. Acute toxicity values were determined using the Probit method. Further, each fipronil toxicity (LC_{10}, LC_{50}, and LC_{90}) value towards *Le. frauenfeldi* was compared with those towards *Li. humile*[14)] and *S. invicta*,[31)] which were calculated using oral acute toxicity data (48 hr) obtained based on the same and/or similar bioassays as was performed in this study. Specifically, given that the lethal concentration values corresponding to four *Li. humile* populations with different genetic structures had been previously reported by Hayasaka *et al.*,[14)] all the acute toxicity values for this species were used as comparative data. The statistical differences between these species with respect to fipronil sensitivity were determined using the *CompParm* function of the 'drc' package in R.[33)] This function implements *t*-test for parameter differences (LC_{50} population X–LC_{50} population Y) that were compared relative to 0. However, *t*-test for *S. invicta* was performed using the same *CompParm* function, but with summary statistics owing to the absence of raw data on acute toxicity.

Results

The 48-hr acute toxicity values (LC_{10}, LC_{50}, and LC_{90}) of fipronil to *Le. frauenfeldi* workers were 35.96, 457.72, 5825.56 μg/L, respectively (Fig. 1, Table 1). Further, its lethal median concentration (LC_{50}) towards *Le. frauenfeldi* workers was significantly lower than that towards *Li. humile* (271–2782 μg/L)[14)] and *S. invicta* (2510 μg/L)[31)] ($p<0.001$). In particular, its toxicity towards the main *Li. humile* population in Japan, which is the most inva-

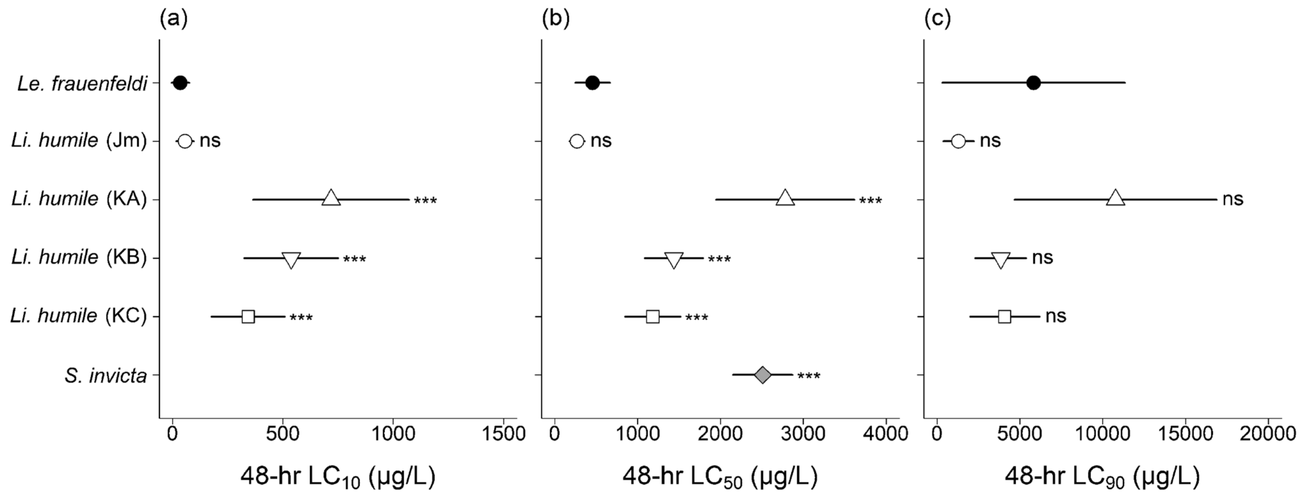

Fig. 1. Comparison of fipronil susceptibility (48-hr LC_{10} (a), LC_{50} (b), and LC_{90} (c) in μg/L) between *Lepisiota frauenfeldi* and two other invasive ant species (*Linepithema humile*, including four genetically different populations[14] and/or *Solenopsis invicta*[31]) using the *CompParm* function in R package, 'drc.' Asterisks and ns indicate significant differences between *Le. frauenfeldi* and the two other invasive ant species (***, $p<0.001$; ns, $p>0.05$). Abbreviations for the different species are shown in Table 1. Symbols: *Le. frauenfeldi* (●); *Li. humile* (Jm: ○, KA: △, KB: ▽, KC: □); *S. invicta* (◇). The oral acute toxicity bioassay procedures for the different test species were the same and/or similar.

sive, but have the highest fipronil sensitivity[14] and towards *Le. frauenfeldi* were statistically similar ($p>0.05$) (Fig. 1b). Similarly, *Le. frauenfeldi* showed the lowest LC_{10} value with respect to fipronil the different among species ($p<0.001$) (Fig. 1a). Conversely, no significant differences were observed between *Le. frauenfeldi* and *Li. humile* populations with respect to their LC_{90} values ($p>0.05$) (Fig. 1c).

Discussion

In this study, the LC_{10} and LC_{50} values of fipronil against *Le. frauenfeldi* were almost the same as those against the main *Li. humile* population in Japan, which is the most invasive, but shows the highest fipronil susceptibility.[14] Further, its LC_{50} was five times lower than that of *S. invicta*[31] (Fig. 1a, b, Table 1). However, the LC_{90} value of *Le. frauenfeldi* was approximately the same as that corresponding to the *Li. humile* population (Fig. 1c). Incidentally, the sensitivity of invasive *Li. humile* populations to fipronil was relatively higher and/or similar to those of other arthropods, including other ants, cockroaches, and isopods.[14] This means that *Le. frauenfeldi* workers can be more sensitive to the insecticide than non-target organisms as well. Therefore, recent eradication/control programs using slow-acting fipronil against *Li. humile* and *S. invicta* can also be effective enough against *Le. frauenfeldi*. The relatively small differences between the fipronil sensitivities (within one order of magnitude in LC_{50}, Table 1) of these three species of ants may be due to their similar body sizes (*Le. frauenfeldi*, 2.5–4.0 mm[24]; *Li. humile*, 2.56–2.72 mm[14]; *S. invicta*, 2.5–6.0 mm[19]). Body size/mass dependence of toxicant sensitivity has been reported although there are exceptions.[34–36] A significant correlation between fipronil toxicity and body length has also been reported for aquatic arthropods.[37]

Again, slow-acting toxic baits are likely to efficacious against invasive ants after their administration because the baits can be

Table 1. Different acute toxicity (48-hr LC_{10}, LC_{50}, and LC_{90} in μg/L) of fipronil among the three invasive ant species (*Lepisiota frauenfeldi*, *Linepithema humile*, and *Solenopsis invicta*). The oral acute toxicity bioassay procedures for the different test species were the same and/or similar.

Species	Range of conc. tested (μg/L)	No. of conc.	48-hr LC_x values [μg/L (95% CI)]			Reference
			LC_{10}	LC_{50}	LC_{90}	
Lepisiota frauenfeldi (*Le. frauenfeldi*)	97.65–6250	7	$35.96_{(-4.84–76.76)}$	$457.72_{(247.04–668.39)}$	$5825.56_{(311.40–11339.71)}$	This study
Linepithema humile populations[a] (*Li. humile*)						Hayasaka *et al.*[14]
Japanese main (Jm)	62.5–1000	5	$57_{(17–96)}$	$271_{(180–362)}$	$1295_{(362–2229)}$	
Kobe A (KA)	156.25–10000	7	$718_{(364–1071)}$	$2782_{(1947–3617)}$	$10776_{(4674–16877)}$	
Kobe B (KB)	156.25–1000	7	$537_{(342–751)}$	$1437_{(1085–1790)}$	$3844_{(2307–5381)}$	
Kobe C (KC)	156.25–5000	6	$343_{(177–509)}$	$1183_{(849–1516)}$	$4081_{(1985–6177)}$	
Solenopsis invicta (*S. invicta*)	1000–10000	6	—	$2510_{(2150–2870)}$	—	Xiong *et al.*[31]

[a] Indicates populations with different genetic structures[39]

brought back to the nest by workers and then shared with nest mates, such as queens and broods.[38] However, given that studies on the horizontal transfer process of slow-acting insecticides in ant nests[13,17] are limited, a further understanding of the efficacy of fipronil is required.

Despite the aforementioned high insecticidal effect of fipronil on invasive *Le. frauenfeldi*, its adverse ecological impacts on native organisms and the environment still need to be considered; thus, environmentally friendly eradication/control strategies for *Le. frauenfeldi* are preferred. Indiscriminatory fipronil susceptibility has been observed regardless of inter- and intra-species.[14] Conversely, given the high sensitivity of invasive ants, including *Le. frauenfeldi* to fipronil compared to non-target organisms,[14] it would be better to design baits of specific fipronil dosages targeting only *Le. frauenfeldi*. With this strategy, *Le. frauenfeldi* would be controlled and/or exterminated effectively with causing low ecological damage to other native species.

Acknowledgements

We thank Dr. Takuo Sawahata for valuable technical advice. We also express our gratitude to Kota Kohara for helping with field sampling. Furthermore, the authors thank Editage (www.editage.com) for English language editing. Part of this study was supported by the Japan Society for the Promotion of Science (JSPS), KAKENHI (grant numbers JP16K15080, JP19K06098, and JP20J12743). The paper benefitted from the constructive comments of two anonymous reviewers.

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