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

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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 LC10 and LC50 values of fipronil for *Le. frauenfeldi* were significantly lower than the previously reported values for *Li. humile* and/or *S. invicta*, and its LC90 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 species2; 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 Goka12) reported that fipronil has high insecticidal effects on *S. invicta* (48-hr LD90: 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-
a wide range of effects on arthropods, and it also eventually af-

Oral acute toxicity bioassays of fipronil in

In brief, commercial fipronil [Prince Flowable, fipronil/water, Osaka, Fukuoka, Kagoshima, and Okinawa Prefectures.24,25] Fur-

Prefecture and Tokyo in 2017, and by 2022 it was detected in

Le. frauenfeldi has been listed as an invasive alien species (IAS)

3. Statistical analyses
All statistical analyses were conducted using R software version

4.2.1.32) The lethal concentration (LC10, LC50, and LC90) 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 (LC10, LC50, and LC90) value towards Le. frauenfeldi was compared with those towards Li. humile14) and S. invicta,31) which were calculated using oral acute toxicity data (48hr) 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 popula-

tions with different genetic structures had been previously re-

ported 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.31) This function implements t-test for parameter differenc-
es (LC50 population X–LC50 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 (LC10, LC50, and LC90) 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 (LC50) 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-

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 tar-
geted. In this study, Le. frauenfeldi workers were sampled from the Tobishima wharf (30°02′03.7″, 136°49′20.4″E), Nagoya City, Aichi Prefecture in October 2017, before their designa-

tion 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 fol-

lowing dry cotton filled with 25% sucrose solution for three days to allow for acclimation to the environment. This was then fol-

owed by the acute toxicity tests. It is also worth noting that the tested individuals were reared in an incubator (LH-30-8CT, Nip-

pon Medical & Chemical Instruments Co., Ltd., Osaka) main-

tained 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 work-
ers 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 cm³) 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). Further-

more, in this study, the nominal concentration of fipronil var-
iplied in the infested area. 21) Additionally, it can negatively impact agricultural and horticultural activities via “ant-Sternorrhyncha (particularly, scale insects which are one of the major agricultural pests) mutualisms”21) 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 sen-
sitivity of Le. frauenfeldi was compared with those of two other known invasive ants (Li. humile and S. invicta).14,31)
sive, but have the highest fipronil sensitivity\(^{14}\) and towards \textit{Le. frauenfeldi} were statistically similar (\(p > 0.05\)) (Fig. 1b). Similarly, \textit{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 \textit{Le. frauenfeldi} and \textit{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 \textit{Le. frauenfeldi} were almost the same as those against the main \textit{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 \textit{S. invicta}\(^{31}\) (Fig. 1a, b, Table 1). However, the LC\(_{90}\) value of \textit{Le. frauenfeldi} was approximately the same as that corresponding to the \textit{Li. humile} population (Fig. 1c). Incidentally, the sensitivity of invasive \textit{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 \textit{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 \textit{Li. humile} and \textit{S. invicta} can also be effective enough against \textit{Le. frauenfeldi}. The relatively small differences between the fipronil sensitivities (within one order of magnitude in LC\(_{50}\)) of these three species of ants may be due to their similar body sizes (\textit{Le. frauenfeldi}, 2.5–4.0 mm\(^{24}\); \textit{Li. humile}, 2.56–2.72 mm\(^{14}\); \textit{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 \(\mu g/L\)) of fipronil among the three invasive ant species (\textit{Lepisiota frauenfeldi}, \textit{Linepithema humile}, and \textit{Solenopsis invicta}). The oral acute toxicity bioassay procedures for the different test species were the same and/or similar.

| Species                  | Range of conc. tested (\(\mu g/L\)) | No. of conc. | 48-hr LC\(_{x}\) values [\(\mu g/L\) (95% CI)] | Reference                  |
|--------------------------|-----------------------------------|--------------|---------------------------------------------|----------------------------|
| \textit{Lepisiota frauenfeldi} (\textit{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 |
| \textit{Linepithema humile} populations\(^{d}\) (\textit{Li. humile}) | 62.5–1000 | 5 | 57(17–96) | 271(183–362) | 1295(562–2229) | Hayasaka et al.\(^{14}\) |
| Japanese main (Jm) | 156.25–10000 | 7 | 57(17–96) | 271(183–362) | 1295(562–2229) | Hayasaka et al.\(^{14}\) |
| Kobe A (KA) | 156.25–10000 | 7 | 718(106–1071) | 2782(1947–3617) | 10776(874–14877) | Hayasaka et al.\(^{14}\) |
| Kobe B (KB) | 156.25–10000 | 7 | 537(142–751) | 1437(1083–1790) | 3844(2307–3381) | Hayasaka et al.\(^{14}\) |
| Kobe C (KC) | 156.25–5000 | 6 | 343(177–509) | 1183(849–1516) | 4081(1985–6177) | Hayasaka et al.\(^{14}\) |
| \textit{Solenopsis invicta} (\textit{S. invicta}) | 1000–10000 | 6 | — | 2510(1190–2870) | — | Xiong et al.\(^{31}\) |

\(^{d}\) Indicates populations with different genetic structures\(^{30}\)
brought back to the nest by workers and then shared with nest mates, such as queens and broods. However, given that studies on the horizontal transfer process of slow-acting insecticides in ant nests 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. Conversely, given the high sensitivity of invasive ants, including *Le. frauenfeldi* to fipronil compared to non-target organisms, it would be better to design baits of specific fipronil dosages to fipronil compared to non-target organisms, and some non-target ground arthropods. As a result of community-wide management targeting the invasive white-footed ant, *Technomyrmex brunneus*, Pest Manag. Sci. 78, 4083–4091 (2022).

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