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A Surprisingly Non-attractiveness of Commercial Poison Baits to Newly Established Population of White-Footed Ant, *Technomyrmex brunneus* (Hymenoptera: Formicidae), in a Remote Island of Japan

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Abstract

The white-footed ant, *Technomyrmex brunneus*, was newly introduced and established in a remote island of Japan and has caused unacceptable damage to the daily life of residents. To establish proper control measures, the present study investigated whether *T. brunneus* is effectively attracted to commercially available poison baits used to exterminate common household pest ants and the Argentine ant in Japan. Cafeteria experiments using three types of nontoxic baits and eight types of commercial poison baits for ants were conducted in the field, and the attractiveness was compared among the baits. The liquid poison bait "Arimetsu," which consists of 42.6% water, 55.4% sugar, and 2.0% borate, and nontoxic 10% (w/v) sucrose water showed the highest attractiveness. On the other hand, other commercial poison baits were not as attractive. Therefore, sucrose liquid is the most effective attractive component to use in poison baits for *T. brunneus*.

Introduction

The white-footed ant, *Technomyrmex brunneus* Forel, 1895, is widespread in South, Southeast, and East Asia, which includes India, Sri Lanka to Indochina, Indonesia, Malaysia, New Guinea, Taiwan, China, the Korean Peninsula, and Japan. In Japan, the species has been recorded in the Ryukyus (including the Daito Islands), Ogasawara Islands (Chichi-jima, Ototo-jima and Ani-jima), Volcano Islands (Iwo-jima), Minami-tori-shima, Kyushu (Kagoshima Prefecture, Aoshima), Shikoku (southern part), Izu Islands (Hachijo-jima), and Kanagawa Prefecture (Yokohama: Naka-ku: Kamome-cho) (Terayama, 2020; Terayama et al., 2014, 2018). Additionally, it has been found indoors in Shimoda City, Shizuoka Pref. (greenhouse); Futtsu City, Chiba Pref. (greenhouse); and Taito-ku, Tokyo (Ueno Zoo: Vivarium of amphibians and reptiles) (Sakamoto et al., 2011).

The Japanese populations of this species (Japanese common name: Ashijiro-hirafushi-ari) had been referred to as *Technomyrmex albipes* (F. Smith, 1861) for a long time. However, in his world revision of the genus *Technomyrmex*, Bolton (2007) revived the status of "*T. albipes* subsp. *brunneus*" as a full species, and discriminated it morphologically from *T. albipes*. By following his revision, "Ashijiro-hirafushi-ari" or "*Technomyrmex albipes*" studied previously in Japan are likely to be reidentified as *Technomyrmex brunneus* (Terayama et al., 2014; Yamane et al., 2018).

Recently, the range of *T. brunneus* has expanded in Japan. In Kyushu, it was recorded in Aoshima (Miyazaki Prefecture) before World War II (Teranishi, 1929), and the population was confirmed to be established later (Ogata, 1995). The southern part of Kagoshima City had been recognized as the northern limit of the *T. brunneus* range in mainland Kyushu. However, around 2001, the southern Kagoshima

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population suddenly began to expand northward, and as of 2007, *T. brunneus* was established in the lowlands of northwestern Kagoshima Prefecture (Shimana & Yamane, 2007). In the Ogasawara Islands, *T. brunneus* was established on Chichi-jima Island (Sugiura, 2008; misidentified as *T. albipes*), and recently, the species was also discovered on Ototo-jima Island (Shimano et al., 2018).

In the Izu Islands, Ogura et al. (2017) formally reported the establishment of a *T. brunneu*s population on Hachijo-jima Island. The authors found that the population density in four of five villages was quite high, and pointed out that a single "super colony" occupied the island. On the other hand, around 2011, the islanders noticed the "outbreak" and the damage inflicted by this species (Hachijo Town Hall: Residents' Section, unpublished). Colonies of *T. brunneu*s nested around houses in the villages and often invaded resident homes to build nests. As a result, not only did the invasions cause discomfort to the residents but the ants also damaged and raided food items. The repeated invasions forced residents to incur heavy expenses to exterminate the ants using commercial pesticides. Furthermore, colonies nesting in electrical devices, such as switchboards and air conditioners, have caused mechanical damage in various parts of the island and have forced owners to suffer expensive repair costs (Hachijo Town Hall: Residents Section, unpublished data). Therefore, Hachijo Town decided to implement control methods for *T. brunneu*s beginning in the 2020 fiscal year.

In several ant species, nutrient exchange among individuals is performed via mouth-to-mouth transfer (trophallaxis); thus, poison bait methods utilizing this behavior are generally used to control invasive ants (Hoffmann et al., 2016). Foraging worker ants carry poison bait (a mixture of the attractant and the insecticidal component) to their nests and pass it to other individuals (workers, queens, and larvae) directly or via trophallaxis. As a result, the insecticide spreads throughout the colony and kills the entire colony. However, *T. brunneu*s has a combination of ecological features that are not seen in other notorious pest ant species such as the fire ant and the Argentine ant. The characteristics of *T. brunneu*s are as follows: they are highly polydomous, nesting on the ground and in trees; they are highly polygynous, with many fertilized permanently wingless worker-like queens, so-called ergatoid queens, in a colony (Yamauchi et al., 1991; Tsuji et al., 1991; Tsuji & Yamauchi, 1994); and nutrient transfer from adults to other colony members (workers, queens, and larvae) is achieved exclusively by specialized trophic eggs (Yamauchi et al., 1991). Therefore, to establish proper control measures for *T. brunneu*s, some issues need to be considered because of their ecological features. In addition, knowledge of related species may not always apply to this species. For example, there is no literature on *T. brunneus* food preferences.

The present study aims to confirm whether the Hachijo-jima population of *T. brunneus* is effectively attracted to commercially available poison baits that are used to exterminate common native pest ants, as well as the invasive Argentine ant that occurs in Japan.

Materials and Methods

Study sites

Hachijo-jima Island has five villages (Mitsune, Okago, Kashitate, Nakanogo, and Sueyoshi), where most residents live. The first trial of the cafeteria experiment (trial 1) was conducted on June 20, 2020, at nine sites: two sites in Mitsune, three sites in Okago, one site in Kashitate, two sites in Nakanogo, and one site in Sueyoshi (filled circles in Fig 1). The second trial (trial 2) was conducted on July 11, 2020, at nine sites: one site in Mitsune, three sites in Okago, two sites in Kashitate, one site in Nakanogo, and two sites in Sueyoshi (unfilled circles in Fig 1).

Cafeteria experiment

One bait series was set at each of the nine sites. Each bait was placed on a sheet of white drawing paper (100 mm in diameter), and the papers were set approximately 100 mm apart from each other. In the first trial conducted on June 20 (hereafter referred to as "trial 1"), the bait series consisted of four commercial poison baits [Fumakilla Ultra Suno Ari Fumakilla (USF), Kincho Ariyou Combat (AC), Earth Garden Hyper Arinosu Korori (HAK), and Arimetsu (AM)] and three non-poison baits [i.e., 10% (w/v) sucrose water (SW), 10% (w/v) honey water (HW), and peanut cream (PC; https://www.sonton.co.jp/products/familycup/)]; filtered tap water (FTW) was used as a negative control (Table 1). AM (liquid poison bait), 10% sucrose water, 10% honey water, and filtered tap water were soaked separately in 40 × 40-mm absorbent cotton squares and placed on the 100-mm-diameter sheets of paper. In the second trial conducted on July 11 (hereafter referred to as "trial 2"), four other commercial poison baits [Ariatol House Sugoto Taiji (AST), Earth Super Arinosu Korori (SAK), InTice Gelanimo Ant Bait (GAB), and Advion Ant Gel (AAG)] were set together with the three non-poison baits and filtered tap water (Table 1). Gel-type baits, i.e., GAB and AAG, were injected separately into 40 × 40-mm square plastic containers (SC Environmental Science Co., Ltd.; https://www.sumika-env-sci.jp/items/detail/2/76/), and placed on the 100-mm-diameter sheets of paper.

Bait series for trials 1 and 2 were set at 09:00 AM, and the 100-mm-diameter sheets of paper were photographed 1 hour and 6 hours later in trial 1, and 1 hour and 3 hours later in trial 2. Ants found on each 100-mm-diameter paper were considered to be attracted, and the number of attracted ants was counted for each bait based on photographs. The total number of attracted ants at the nine sites (for the eight different baits at the two different time windows) were compiled for each trial (Tables 2 and 3).

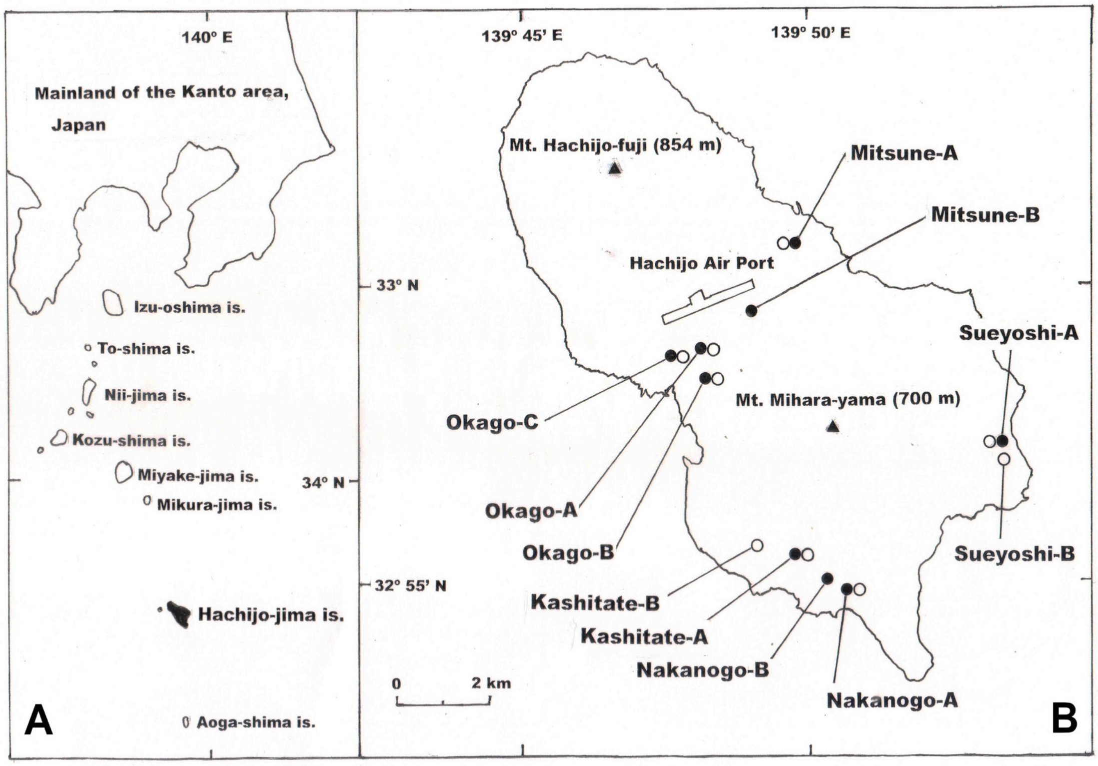

Fig 1. The location of Hachijo-jima Island (in gray color) in the Izu Islands (A), and the locations of the 11 sites where cafeteria experiments were conducted (B). The filled circles represent the sites of trial 1 and the unfilled circles represent the sites of trial 2.

Statistical analyses

Two-way analysis of variance (two-way ANOVA) tests were performed separately for the trial 1 and trial 2 datasets (Tables 2 and 3) to compare the numbers of attracted individuals for each bait and for each time window (Tables 4 and 5). Furthermore, significant main effects were examined using Holm's multiple comparison tests (SRB). Additionally, one-way ANOVA tests were performed as needed. All ANOVA and SRB tests were performed using the software js-STAR 9.8.6j (Tanaka & Nakano, 2018).

Table 1. Details of the commercial poison baits for ants.

Product name **URL**	**Insecticidal component**	**Form**	**Abbreviation**
Trial 1			
Fumakilla Ultra Suno Ari Fumakilla https://fumakilla.jp/insecticide/272/	Fipronil	Paste	USF
Kincho Ariyou Combat https://www.kincho.co.jp/seihin/insecticide/ari/combat_alpha/index.html	Fipronil	Paste	AC
Earth Garden Hyper Arinosu Korori https://www.earth.jp/products/earth-garden-ari-korori/index.html	Fipronil	Paste	HAK
Arimetsu https://www.yokohamaueki.co.jp/arimetsu/	Borate	Liquid	AM
Trial 2			
Ariatol House Sugoto Taiji https://www.sc-engei.co.jp/guide/detail/1481.html	Fipronil Pyriproxyfen	Paste	AST
Earth Super Arinosu Korori https://www.earth.jp/products/ari-korori-super/	Hydramethylnon Dinotefuran	Granule Gel	SAK
InTice Gelanimo Ant Bait https://www.rockwelllabs.com/inticegelanimo.html	Borate	Gel	GAB
Advion Ant Gel https://www.syngentappm.com/advionrant-gel	Indoxacarb	Gel	AAG

Table 2. Number of attracted individuals in trial 1. Abbreviations of the commercial poison baits are given in Table 1; SW, 10% (w/v) sucrose water; HW, 10% (w/v) honey water; PC, peanut cream; FTW, filtered tap water. Abbreviations of the scientific names of non-*Technomyrmex brunneus* species are as follows: Mc, *Monomorium chinense* Santschi, 1925; Pp, *Pristomyrmex punctatus* (F. Smith, 1860). Abbreviations of the time windows are as follows: 1 HA, 1 hour after setting the bait series; 6 HA, 6 hours after setting the bait series. For site locations, see Fig 1.

Sites **Time window**	**USF**	**AC**	**HAK**	**AM**	**SW**	**HW**	**PC**	**FTW**
Mitsune-A								
1 HA	0	1	7	157	96	89	10	4
6 HA	0	0	2	121	33	284	231	10
Mitsune-B								
1 HA	0	0	0	0	41	0	0	0
6 HA	1	3	2	31	173	9	0	0
Okago-A								
1 HA	0	0	7	15	50	34	0	1
6 HA	8	1	7	174	52	14	118	0
Okago-B								
1 HA	0	0	2	5	0	1	0	0
6 HA	1	2	2	225	26	20	78	1
Okago-C								
1 HA	0	0	1	37 (Mc 30)	18	30	5	1 (Pp 8)
6 HA	2	0	1	83	55	38	46	1
Kashitate-A								
1 HA	2	0	7	377	89	41	4	7
6 HA	11	1	6	238	499	141	73	1
Nakanogo-A								
1 HA	0	0	0	29	14	4	3	1
6 HA	0	1	1	38	36	109	4	5
Nakanogo-B								
1 HA	0	0	0	5	4	0	2	0
6 HA	0	0	0	87	18	1	50	0
Sueyoshi-A								
1 HA	0	0	0	146	207	87	1	3
6 HA	79	8	82	729	66	62	19	19

Results

Trial 1

Table 2 shows the number of attracted individuals at the two different time windows, i.e., 1 hour (1 HA) and 6 hours (6 HA) after setting the bait series. *Monomorium chinense* (Mc) and *Pristomyrmex punctatus* (Pp), as well as *T. brunneus*, were attracted to the bait series. Of the three ant species, *T. brunneus* is extremely dominant in all locations, while the number of attracted individuals varied widely depending on the locations and time windows; the standard deviation was large for each bait (Table 3).

The results of the two-way ANOVA showed no significant difference in the number of attracted *T. brunneus* individuals between the time windows at the 5% level ($F(1, 7) = 4.36$, $0.10 > p > 0.05$), but there were significant differences among the baits at the 1% level ($F(7, 7) = 7.33$, $p < 0.01$). Significant differences in the mean number of attracted *T. brunneus* individuals were observed between the following combinations at the 5% level: USF < AM, AC < AM, HW < AM, PC < AM, FTW < AM, and AC < sucrose water (SW).

Table 3. The mean and standard error of the number of attracted individuals of *Technomyrmex brunneus* in trial 1. The total number of individuals attracted to each bait is given in parentheses. Abbreviations of the baits and time windows are given in Tables 1 and 2. The superscripts (a, b, and c) of the baits indicate significant differences at the 5% level by Holm's multiple comparison tests following analysis by two-way ANOVA.

Baits	**1 HA**	**6 HA**
USF ^{b}	0.22 ^{a} ± 0.63 (2)	11.33 ^{a} ± 34.12 (102)
AC $^{a, b}$	0.11 ^{a} ± 0.31 (1)	1.78 ^{a} ± 2.39 (16)
HAK ^{b}	2.67 ^{a} ± 3.13 (24)	11.45 ^{a} ± 25.04 (103)
AM ^{c}	85.67 ^{b} ± 117.63 (771)	191.78 ^{b} ± 202.62 (1726)
SW $^{b, c}$	57.67 ^{a} ± 62.16 (519)	106.45 ^{a} ± 145.46 (958)
HW ^{b}	31.78 ^{a} ± 33.59 (286)	75.33 ^{a} ± 86.48 (678)
PC ^{b}	2.78 ^{a} ± 3.20 (25)	68.78 ^{a} ± 67.59 (619)
FTW ^{b}	1.89 ^{a} ± 2.23 (17)	4.11 ^{a} ± 6.12 (37)
ANOVA results		
F	4.22	4.01
df	7	7
p	<0.01	<0.01

In addition, A × B, which indicates the interaction between factor A (time window) and factor B (bait), was not significantly different at the 5% level ($F (7, 7) = 1.06$, N.S.). Attractiveness was tested with ANOVA after dividing the trial 1 dataset into two sub-datasets by the two different time windows. The results showed significant differences at the 1% level in both the sub-datasets [1 HA: SS = 67,647.875, df = 7, MS = 9664.125, $F = 4.22$ ($p < 0.01$); 6 HA: SS = 279,785.097, df = 7, MS = 39,696.300, $F = 4.01$ ($p < 0.01$)]. The combinations in which a significant difference was observed at the 5% level were USF < AM, AC < AM, HAK < AM, PC < AM, PW < AM in 1 HA, and USF < AM, AC < AM, HAK < AM, PW < AM in 6 HA.

From the above results, the bait that showed the highest attractiveness in trial 1 was Arimetsu (AM); 10% SW also showed a similar attractiveness. In contrast, the attractiveness of the paste-form poison baits (USF, AC, and HAK) was low, and when compared with the mean number of attracted *T. brunneus* individuals, those were less than 5% of the attractiveness of Arimetsu.

Trial 2

Table 3 shows the number of attracted individuals at the two different time windows, i.e., 1 hour (1 HA) and 3 hours (3 HA) after setting the bait series. *Tetramorium bicarinatum* (Nylander, 1846) (Tb), *M. chinense* (Mc), *P. punctatus* (Pp),

Table 4. Number of attracted individuals in trial 2. Abbreviations of the commercial poison and non-poison baits are given in Tables 1 and 2. Abbreviations of the scientific names of non-*Technomyrmex brunneus* species are as follows: Tb, *Tetramorium bicarinatum*; Ps, *Paraparatrechina sakurae*; Cv, *Camponotus vitiosus* (for others, see Table 2). Abbreviations of the time windows are as follows: 1 HA, 1 hour after setting the bait series; 3 HA, 3 hours after setting the bait series. For site locations, see Fig 1.

Sites Time window	AST	SAK	GAB	AAG	SW	HW	PC	FTW
Mitsune-A								
1 HA	0	10	0	0	1	0	0	0
3 HA	0	1	0	0	1	0	0	2
Okago-A								
1 HA	2	0	2	0	117	60	21	3
3 HA	2	9	80	19	416	486	5	5
Okago-B								
1 HA	0	0	0	1	0	0	0 (Mc 1)	0
3 HA	1	14	60	34	50	4	1	0
Okago-C								
1 HA	0	0	0	0	0	1	0	0
3 HA	10	37	76	54	46	12	17 (Ps 1)	4
Kashitate-A								
1 HA	0	4	18	0	78	28	1 (Tb 1)	0
3 HA	2	2	20	21	206	102	0 (Tb 52)	0
Kashitate-B								
1 HA	7	4	1	1	35	6	0	0
3 HA	12	5	33	45	27	4	0	0
Nakanogo-A								
1 HA	0	0 (Ps 1)	0	0	0 (Pp 1)	0	0 (Mc 4)	0
3 HA	1	0 (Pp 1)	1 (Pp 2)	0	18 (Mc 36)	1 (Pp 1)	0 (Mc 66)	0
Sueyoshi-A								
1 HA	0	19	12	55	210	61	1	0
3 HA	1	50	170	57	282	45	0	0
Sueyoshi-B								
1 HA	0 (Cv 1)	0 (Pp 4)	0	0	0	0	0	1
3 HA	0	0 (Pp 68)	0 (Pp 1)	0	13	14	0	0

Paraparatrechina sakurae (Ito, 1914) (Ps), and *Camponotus vitiosus* (F. Smith, 1874) (Cv), as well as *T. brunneus*, were attracted to the bait series. Of the six ant species, *T. brunneus* is extremely dominant in all locations, while the number of *T. brunneus* individuals varied widely depending on the locations and time windows; the standard deviation was large for each bait (Table 5).

Table 5. The mean and standard error of the number of attracted individuals of *Technomyrmex brunneus* in trial 2. The total number of individuals attracted to each bait is given in parentheses. Abbreviations of the baits and time windows are given in Tables 1, 2, and 4. The superscripts (a and b) of the baits indicate significant differences at the 5% level by Holm's multiple comparison tests following analysis by two-way ANOVA.

Baits	1 HA	3 HA
AST ^{a}	1.00 ^{a} ± 2.21 (9)	3.00 ^{a} ± 3.80 (27)
SAK ^{a}	4.11 ^{a} ± 6.15 (37)	13.11 ^{a} ± 17.09 (118)
GAB ^{a}	3.67 ^{a} ± 6.25 (33)	37.78 ^{a} ± 32.18 (340)
AAG ^{a}	6.33 ^{a} ± 17.21 (57)	23.33 ^{a} ± 23.11 (210)
SW ^{b}	49.00 ^{b} ± 69.51 (441)	117.67 ^{b} ± 139.94 (1059)
HW $^{a, b}$	17.33 ^{a} ± 24.58 (156)	74.22 ^{a} ± 148.85 (668)
PC ^{a}	2.56 ^{a} ± 6.53 (23)	2.56 ^{a} ± 5.34 (23)
FTW ^{a}	0.44 ^{a} ± 0.96 (4)	1.22 ^{a} ± 1.87 (11)
ANOVA results		
F	3.67	3.02
df	7	7
p	<0.01	<0.05

The results of the two-way ANOVA showed no significant difference in the number of attracted *T. brunneus* individuals between the time windows at the 5% level ($F(1, 7) = 2.77$, N.S.), but there were significant differences among the baits at the 1% level ($F(7, 7) = 4.98$, $p < 0.01$). Significant differences in the mean number of attracted *T. brunneus* individuals were observed between the following combinations at the 5% level: AST < SW, SAK < SW, GAB < SW, AAG < SW, PC < SW, and FTW < SW. In addition, A × B, which indicates the interaction between factor A (time windows) and factor B (baits), was not significantly different at the 5% level ($F(7, 7) = 1.19$, N.S.). The attractiveness was tested by ANOVA after dividing the trial 2 dataset into two sub-datasets by the two different time windows. The results showed significant differences at the 1% level in the 1 HA sub-dataset and at the 5% level in the 3 HA sub-datasets [1 HA: SS = 16,994.444, df = 7, MS = 2427.777, F = 3.67 ($p < 0.01$); 3 HA: SS = 110,984.388, df = 7, MS = 15,854.912, F = 3.02 ($p < 0.05$)]. The combinations in which a significant difference was observed at the 5% level were AST < SW, SAK < SW, GAB < SW, AAG < SW, PC < SW, and FTW < SW in 1 HA, and AST < SW, SAK < SW, PC < SW, and FTW < SW in 3 HA.

From the above results, no bait exceeded the attractiveness of 10% SW in trial 2. In contrast, the attractiveness of the gel-form poison baits (GAB and AAG) was low, and when compared with the mean number of attracted *T. brunneus* individuals, those were less than 32% of the attractiveness of SW.

Discussion

The results of the two trials of the cafeteria experiment conducted on Hachijo-jima Island revealed that SW effectively attracts *T. brunneus*. Among the commercial poison baits for ants, only Arimetsu showed a strong attractiveness, equivalent to or more than that of SW. Arimetsu consists of 42.6% water, 55.4% sugar (attracting component), and 2.0% borate (insecticidal component). *Technomyrmex difficilis* Forel, 1892, belonging to the *T. albipes* species group in North America (Florida), is known to be attracted strongly to sugar-rich baits, but is less attracted to protein- and lipid-rich baits (Klotz et al., 2008). Based on indoor experiments conducted by Warner and Scheffrahn (2005) and Warner et al. (2005), sucrose-enhanced liquid baits are recommended to effectively attract *T. difficilis* (misidentified as *T. albipes* in the paper). Warner and Scheffrahn (2005) reported that *T. difficilis* is attracted most strongly to 25%–40% SW. On the other hand, the present study revealed that paste-form poison baits containing fipronil, which is often used to exterminate common native ants, as well as fire ants and the Argentine ant in Japan, cannot effectively attract *T. brunneus*. Therefore, from the viewpoint of attractiveness to *T. brunneus*, the substrate of the bait suitable for *T. brunneus* would be a liquid composed mainly of sugar (sucrose), or a biodegradable sponge or hydrogel containing such liquid.

The insecticidal component of Arimetsu is borate. However, in experiments with *T. difficilis*, Warner and Scheffrahn (2005) found that thiamethoxam and imidacloprid of neonicotinoid showed a higher insecticidal effect than fipronil and borate when sucrose solution or a similar liquid was used as the substrate. Additionally, there are no successful cases in which borate was used to eradicate highly invasive ant populations (Hoffmann et al., 2016). The effectiveness of insecticidal components is known to differ depending on the ant species. For example, among the insecticide components used commonly, hydramethylnon controls *Monomorium pharaonis* (Linnaeus, 1758) and *Linepithema humile* (Mayr, 1868) effectively, but controls *Tapinoma melanocephalum* (Fabricius, 1793) less effectively (Klotz et al., 1996; Ulloa-Chacón & Jaramillo, 2003). Therefore, the confirmation of an insecticidal component suitable for *T. brunneus* will be an important issue in future studies. As *T. brunneus* individuals exchange nutrients with each other exclusively through specialized trophic eggs (Yamauchi et al., 1991), it is also quite important to know how this behavioral feature influences the diffusion efficiency of insecticidal components in the nest.

Thus, from the viewpoint of the attractant and insecticidal bait components, it is clear that conventional extermination protocols used for fire and Argentine ants cannot be applied directly to *T. brunneus*. Along with the development of baits suitable for *T. brunneus*, it is necessary to establish a comprehensive control protocol specialized for the Hachijo-jima population, such as the establishment of bait installation methods, environmental improvement methods, and local quarantine measures, according to the characteristics of the *T. brunneus* life history under the local bioclimate and other environmental conditions on Hachijo-jima Island.

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Authors' contributions

MT: conceptualization, methodology, formal analisys, writing
RF: conceptualization, methodology, investigation, writing
TO: conceptualization, methodology, investigation, writing
ES: conceptualization, methodology, writing
KE: conceptualization, methodology, writing, project administration (coordination of government-academia joint research).

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