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Running title: Hydrogel bait for community-wide white-footed ant management

Development of an effective hydrogel bait and an assessment of community-wide management targeting the invasive white-footed ant, *Technomyrmex brunneus*

Eiriki Sunamura,[ab†]* Mamoru Terayama,[b†] Ryota Fujimaki,[c] Takashi Ono,[c] Grzegorz Buczkowski[d‡] and Katsuyuki Eguchi[be‡]

* Correspondence to: Department of Forest Entomology, Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki 305-8687, Japan. E-mail: esunamura@ffpri.affrc.go.jp

[a] Department of Forest Entomology, Forestry and Forest Products Research Institute, Matsunosato 1, Tsukuba, Ibaraki 305-8687, Japan

[b] Graduate School of Science, Tokyo Metropolitan University, Minami-osawa 1-1, Hachioji, Tokyo 192-0397, Japan

[c] Hachijo Town Hall, Okago 2551-2, Hachijo, Tokyo 100-1498, Japan

[d] Department of Entomology, 901 W. State St., Purdue University, West Lafayette, IN 47907, USA

[e] Department of International Health and Medical Anthropology, Institute of Tropical Medicine, Nagasaki University, Nagasaki, 852-8523, Japan

[†,‡] These authors contributed equally to this work

ABSTRACT

BACKGROUND: Ants are one of the most serious household pests. White-footed ants in the genus *Technomyrmex* (Hymenoptera: Formicidae) are invasive species of increasing global importance as evidenced by recent range expansion, nuisance pest status in residential areas, and control difficulties driven mainly by lack of effective bait products. The goal for the current study was to develop an effective hydrogel bait and community-wide management program for controlling the invasive *Technomyrmex brunneus* in a residential area of Hachijo Island, Japan.

RESULTS: In laboratory insecticide screening, hydrogel baits containing thiamethoxam achieved higher *T. brunneus* mortality than those containing dinotefuran, imidacloprid, boric acid, or fipronil at the concentrations tested. Hydrogel baits containing 0.01 and 0.001% thiamethoxam resulted in ≥90% mortality within 7 days. However, horizontal transfer effect was not strong with either concentration. Field experiments confirmed that hydrogel baits containing 10% and 30% sugar are highly attractive to *T. brunneus*. The community-wide treatment utilizing hydrogel bait containing 0.001% thiamethoxam and 30% sugar significantly suppressed *T. brunneus*. Town residents were given the opportunity to participate in the program by applying hydrogel baits around their homes, and a follow-up survey revealed that the residents regarded hydrogel baits as easy to apply and highly attractive and effective against *T. brunneus*.

CONCLUSION: The hydrogel bait and management program developed in this study can be used to suppress *T. brunneus*. Furthermore, our results demonstrate that a large network of highly motivated and properly trained members of the public can be highly effective in managing invasive ant populations.

Keywords: arboreal ant; biological invasions; invasive ants; neonicotinoid; nuisance pest; subtropical island

1 INTRODUCTION

Invasive ants have complex, multi-level impacts on the environment, economy, and human well-being.[1, 2] In urban areas, invasive ants frequently possess high reproduction, are comprised of large multi-nest and multi-queen colonies, and invade buildings in high numbers and frequency.[1] Area-wide treatment using insecticidal bait is the most practical and effective method for controlling invasive ants over relatively large areas.[3] Many invasive ant species prefer liquid, carbohydrate-rich food sources (e.g., nectar and honeydew produced by hemipteran insects);[1] thus, liquid baits are suitable. However, liquid baits require bait stations and the deployment of numerous bait stations over a large area is cost prohibitive and not practical. Additionally, area-wide treatments to control invasive ants are typically conducted by professionals either through hand or aerial broadcast.[4-8]

To overcome the aforementioned deployment problems associated with conventional liquid baits, a novel insecticide delivery method which relies on hydrogels has been developed.[9, 10] Hydrogels are superabsorbent polymers that readily absorb water and water-soluble materials such as toxicants and feeding stimulants (e.g. sucrose). The hydrogels are then dispersed over the treatment area and allow the ants to feed on the liquid from the hydrogels. Since their development, hydrogel baits have been successfully utilized for the control of the invasive Argentine ant *Linepithema humile*, which prefers liquid carbohydrates.[4, 5, 11, 12] Additionally, hydrogel baits are easy to formulate and apply, relatively inexpensive, and do not require dispensers.[9] A neonicotinoid insecticide, thiamethoxam, has frequently been used in hydrogel baits.[4, 5, 11, 12] Environmental risks of neonicotinoids have been documented,[13,14] but hydrogel baits use less insecticide relative to traditional broadcast sprays.[9]

White-footed ants in the genus *Technomyrmex* (Hymenoptera: Formicidae) are invasive species of increasing global importance. *Technomyrmex difficilis* is thought to be native to Madagascar, but began spreading in southeastern Asia and Oceania more than 70 years ago.[15] More recently, it has invaded Florida and West Indies and is becoming a significant nuisance pest in residential areas.[16, 17] A related species, *T. brunneus* appears to be following a similar pattern. This species was originally distributed in tropical Asia, but invaded Japan's Ryukyu Islands more than 90 years ago, and began to spread rapidly in the country in the 2000s.[18] On Hachijo Island, an oceanic island ca. 300 km south of central Tokyo, a *T. brunneus* outbreak began around 2011,[18] but the species was not identified as *T. brunneus* until 2016.[19] This species is arboreal and inhabits forests, gardens, and other green areas. Residential housing on Hachijo Island is typically surrounded by thick and dense vegetation and/or is built in close proximity to forested areas which facilitates indoor invasions of *T. brunneus* (Figure 1). In response to problems caused by *T. brunneus*, the government of Hachijo Island launched a management program in 2020.[18] However, ant baits available in Japan are not suitable for area-wide use against *T. brunneus*. A previous study showed that granular, paste, and gel baits available in Japan are not attractive to *T. brunneus*.[20] Meanwhile, 10% sugar water and a liquid bait containing 55% sugar were attractive, suggesting that *T. brunneus* prefers liquid foods. Thus, hydrogel baits may be suitable for use against *T. brunneus*.

In addition to the lack of available products, the absence of trophallaxis in *T. brunneus* may limit the efficacy of insecticidal baits. In general, ants exchange nutrients among colony members via trophallaxis.[21, 22] Insecticidal baits aim to exploit trophallaxis to deliver the active ingredient to all colony members, including brood and queens, and efficiently control the whole colony. However, *T. brunneus* does not utilize trophallaxis for the transfer of nutrients among workers and nutritional transfer is exclusively by trophic eggs.[23] Thus, horizontal transfer of insecticides via trophallaxis cannot be expected in *T. brunneus*. In such a case, efficacy of insecticidal baits might be limited.

The goal of the current study was to develop an effective hydrogel bait and community-wide management program for *T. brunneus*. Imidacloprid and thiamethoxam are effective for controlling *T. difficilis*,[16] but the efficacy of various insecticides against *T. brunneus* is unknown. Therefore, the first objective was to formulate various insecticides into hydrogel baits and assess their efficacy against *T. brunneus*. Preliminary tests indicated that a hydrogel bait containing thiamethoxam is effective. The second objective was to examine the potential of thiamethoxam in hydrogel baits to be horizontally transferred within *T. brunneus* colonies. The third objective assessed the palatability of hydrogel bait matrix to field populations of *T. brunneus*. The fourth objective evaluated the feasibility and efficacy of a community-wide management program against *T. brunneus* in a residential area of Hachijo Island using hydrogel baits developed from the outcomes of the first three objectives. We evaluated a unique approach where the public was given the opportunity to participate in a community-wide management program by applying hydrogel baits around their own residences. In Japan, similar trials have been done to suppress the invasive Argentine ant with commercial ant baits, but the efficacy of this method has not been reported.[24]

2 MATERIALS AND METHODS

2.1 Insects

A colony of *T. brunneus* containing multiple queens and approximately 10,000 workers was collected in Kashitate, Hachijo Island on July 2020. This colony was used in all laboratory experiments except for 2.3 low concentration assays (detailed later). In this experiment, another colony collected in Otchogahama, Hachijo Island on September 2021 was used. Each colony was placed in a 38 × 23 × 25 cm plastic box and maintained in original nesting material. The inner wall of the box was coated with Fluon to prevent escapes. The colonies were maintained at 27°C and were fed jelly and dead crickets twice a week and water *ad libitum*.

2.2 Hydrogel bait

Hydrogel baits for laboratory experiments (objective 1 and 2) were prepared by dissolving 30 g of granulated sugar (Nissin Sugar Co., Ltd., Tokyo, Japan) in 70 g deionized water in a beaker and adding the required amount of insecticide (detailed later). Next, 0.5 g of superabsorbent acrylic acid polymer (Newsorb, Newstone International, Tokyo, Japan) was added to the solution and the hydrogel was allowed to fully saturate for approximately 30 min at 25°C.

2.3 Screening of active ingredients

For objective 1, 5 insecticides commonly used in ant baits were incorporated into hydrogels and tested against *T. brunneus*: 0.01% thiamethoxam (FUJIFILM Wako Pure Chemical Corporation, 99.7% purity), 0.01% dinotefuran (FUJIFILM Wako Pure Chemical Corporation, 99.0% purity), 0.01% imidacloprid (FUJIFILM Wako Pure Chemical Corporation, 98.0% purity), 1% boric acid (Kanto Chemical Co., Inc., 99.5% purity), and 0.00052% fipronil (Tokyo Chemical Industry Co., Ltd., >97.0% purity). For fipronil, which is less soluble in water than the other compounds, a saturated aqueous solution was prepared at room temperature (0.7 mg fipronil in 70 g water), residue was removed by filter paper, and the solution was formulated into the hydrogel bait. The concentration of fipronil was determined by HPLC equipped with a LC-20AD pump, CBM-20A system controller, CTO-20AC column oven, Shim-pack Scepter C18 column, and SPM-M20A photodiode array detector (all from Shimadzu Corporation, Kyoto, Japan) and a calibration curve with fipronil standard was generated. Blank bait for controls contained 30% granulated sucrose but no insecticide.

Bioassays were conducted in plastic cups (10 cm diameter × 5.5 cm high). The inner wall of each cup was coated with Fluon to prevent escapes. A filter paper (9 cm diameter) was placed on the bottom of each cup. Twenty *T. brunneus* workers were randomly selected from a laboratory colony and gently placed inside the cup using a brush. The ants were allowed to acclimate to the cup for 24 hours while provided with 1.5 g hydrogel (made from deionized water and Newsorb but no sugar) for hydration. No food was provided during the acclimation period. After acclimation, the ants were provided with 1.5 g hydrogel bait in a vial cap (2 cm diameter × 1 cm high). The number of ants feeding was recorded at 10, 20, 30, 40, 50, 60, 90, and 120 minutes after bait introduction. Ant mortality was recorded at 1, 2, 3, and 7 days. Eight replicates were performed for each insecticide and controls. Thiamethoxam and dinotefuran were highly effective and further testing was performed to assess the efficacy of lower concentrations of these insecticides. Hydrogel baits containing 0.001, 0.0001, and 0.00001% thiamethoxam or dinotefuran were prepared and tested against *T. brunneus* as described above. Eight replicates were tested for each insecticide concentration and controls.

2.4 Evaluation of insecticide horizontal transfer

Results of insecticide screening tests (2.3) demonstrated that thiamethoxam was the most effective insecticide for controlling *T. brunneus*. Laboratory bioassays were performed to assess the potential of thiamethoxam to be horizontally transferred within *T. brunneus* colonies when delivered via hydrogel baits (objective 2). Ten workers (donors) were placed into a Fluon-coated plastic cup (10 cm diameter × 5.5 cm high) containing a thin layer of moist plaster on the bottom. The ants were randomly chosen from a laboratory colony and marked on the abdomen with white oil-based paint using a pen applicator. Ten randomly chosen workers (recipients) were placed into another cup without marking. The donors and recipients were starved for 24 hours. Following starvation, 1.5 g thiamethoxam hydrogel bait was placed inside a vial cap and offered to donor ants. The number of donors feeding on the bait was recorded every 15 min for 2 hours. Following feeding, the donors were transferred to the cup containing the recipients. Interactions among donors and recipients were observed every 15 min for 1 hour. A vial cap containing 1.5 g blank hydrogel bait (30% sugar and no insecticide) was provided to the ants as a food 1 day after introducing the donors. Mortality in the donors (individuals with marking) and the recipients (individuals without marking) was recorded at 1, 2, 3, and 7 days after introducing the donors. Three concentrations of thiamethoxam, 0.01%, 0.001%, and 0% (control) were tested and 7 replicates were performed for each concentration.

2.5 Field palatability of hydrogels

For objective 3, the palatability of hydrogel bait matrix and other food matrices (all without insecticides) to a field population of *T. brunneus* was assessed at Mishima Shrine, Kashitate, Hachijo Island (33.074°, 139.791°) on April 28, 2021. Hydrogel baits were prepared using tap water, superfine sugar (Mitsui Sugar Co., Ltd., Tokyo, Japan), and Newsorb at the weight ratio of 70: 30: 0.5 (30% w/w sugar) and 90: 10: 0.5 (10% w/w sugar). Insecticides were not included, because laboratory experiments for objective 1 and 2 showed that addition of 0.001% thiamethoxam does not reduce the palatability of hydrogel baits.

A bait choice trial (i.e. “cafeteria-style” experiment) offered 7 food sources simultaneously and compared their relative palatability. The food sources were hydrogels containing 10% w/w and 30% w/w sugar, 10% w/w and 30% w/w sugar water (superfine Mitsui Sugar), 10% w/w honey water (lotus honey of Kato Brothers Honey Co., Ltd., Tokyo, Japan), peanut cream (a pasty mixture of peanut butter and syrup containing 25% lipid, 45% carbohydrate, and 5.5% protein; SONTON HOLDINGS CO., LTD., Tokyo, Japan), and tap water (control). The latter 5 food sources were also used in a previous study to compare the palatability of these foods to commercial ant baits.[20] Pieces of white drawing paper (10 cm diameter) were placed on the ground as monitoring stations. Approximately 15 grams of each food was placed on a 4 × 4 cm piece of paraffin paper (4 × 4 cm cotton pad for liquid food sources) assuring that the size and shape of the food item was consistent across the different food sources. The food was then placed on individual station. The stations were placed along a 130 cm transect at 10 cm intervals. Ordinal positions of the 7 foods tested were random. Six replicates were performed for each food at transects spaced 5–10 m apart. The experiment started at 10:00 AM and all stations were photographed at 1 and 3 hours after placement. The number of *T. brunneus* on each station was later counted.

2.6 Field cooperative treatment

The efficacy of hydrogel bait containing 0.001% thiamethoxam was evaluated against a field population of *T. brunneus* in a community-wide management program (objective 4). The project was a coordinated effort between local government officials, public workers in Hachijo Town, and residents of Kashitate Village, with technical guidance, support, and advice from university and government researchers. An area of approximately 75 hectares in Kashitate Village, Hachijo Island was selected for the treatment. The hydrogel bait was prepared 3 days before use. Thiamethoxam (Santa Cruz Biotechnology, Dallas, U.S.A.: ≥98% purity), superfine sugar (Mitsui Sugar), and tap water were poured into 45–80 L plastic buckets and stirred until the ingredients were dissolved. Next, Newsorb was added and the hydrogel was allowed to fully saturate. The mixing ratio of water, sugar, thiamethoxam, and Newsorb was 70: 30: 0.001: 0.5 (42 kg: 18 kg: 0.6 g: 300 g in the case of 80 L bucket) resulting in 0.001% thiamethoxam in 30% w/w sugar water. The bait was placed into 1.9 L plastic containers (ca. 1.8 kg each) and kept at 20–24°C. The containers, together with disposable plastic spoons for bait application, were distributed to homeowners and public workers. All residents received instructions on: (a) proper bait handling and application, (b) amount of product to use and (c) treatment date (May 30, 2021). The residents were instructed via information sessions and community bulletin boards to apply the bait (all of the 1.8 kg provided per residence) around their houses in areas where *T. brunneus* are likely to forage (e.g., near observed ant trails, around house perimeter, under trees and hedges). In total, the bait was applied to 225 private residences, 47 residences for public employees, 18 public facilities, and 2 shrines (Figure 2). This corresponds to a total of ca. 500 kg hydrogel bait including 5 g thiamethoxam.

The efficacy of the treatment was evaluated by quantifying ant abundance in treatment and control plots using cotton pads saturated with sugar water. The surveys were conducted on May 26 (4 days before treatment), June 8 (9 days after treatment), and July 6 (37 days after treatment). Treatment monitoring sites were placed within 10 treated residences (Figure 2), and additional 5 untreated sites (agricultural land or forest edge) ≥200 m away from the treated areas were used as controls. At each site, 10 cotton pads (4 × 4 cm) soaked with ca. 15 g of 10% sugar water were laid on the ground at 2–5 m intervals. The cotton pads were photographed 1 hour after placement and *T. brunneus* present on the pads were later counted. The surveys were performed during daytime. The placement of cotton pads within a site was not always consistent across monitoring surveys.

A follow-up survey was conducted among the residents of Kashitate Village approximately 1 month after the treatment to gauge the resident's attitudes toward the treatment and the results. Survey questions were distributed and anonymous answer sheets collected via the postal service. The questions were as follows: 1) ease of handling of hydrogel bait (5-grade evaluation), 2) attractiveness of hydrogel bait to *T. brunneus* (5-grade evaluation), 3) when *T. brunneus* density became the lowest (X days after treatment), 4) maximum decline of *T. brunneus* density (percent decline from the pre-treatment status), 5) *T. brunneus* density 1 month after treatment (X% change relative to pre-treatment density), 6) any effect on non-target organisms (free-form question), and 7) any additional comments.

2.7 Statistical analyses

All statistical analyses were performed using R Version 4.1.0.[25] For objective 1, screening of active ingredients (2.3), a generalized linear mixed model (GLMM) was used to compare the attractiveness of hydrogel baits containing different insecticides to that of control (R package "lmerTest"). The insecticides, time elapsed, and their interaction were set as the fixed factors, test cup as a random factor, and the number of ants feeding as the response variable. To compare the efficacy of hydrogel baits across treatments, a survival analysis was performed using R package "survminer". Survival curves for individual insecticides were drawn based on the time required for individual ant deaths. Pairwise comparisons were made between the curves in a log-rank test with the Benjamini-Hochberg adjustment. Similarly, a survival analysis was performed to assess the efficacy of low dose thiamethoxam and dinotefuran.

For objective 2 investigating horizontal transfer (2.4), a GLMM was constructed to evaluate the effect of thiamethoxam concentration (0, 0.001, and 0.01%) and time elapsed (8 observations) on the number of ants feeding on hydrogel baits. Test cup was set as a random factor. To analyze the efficacy of hydrogel baits, survival curves for donors and recipients were drawn for each thiamethoxam concentration based on the time required for individual ant deaths. Pairwise comparisons were made between the curves in a log-rank test with the Benjamini-Hochberg adjustment.

For objective 3, the field palatability test (2.5), a GLMM was used to determine if the number of *T. brunneus* varied significantly among foods and across different timings. The food (7 kinds), time elapsed (2 timings), and their interaction were set as the fixed factors, transect as a random factor, and the number of ants attracted as the response variable. In the GLMM, Tukey's multiple comparison tests were performed to test for differences in the palatability of the different foods (R package "multcomp").

For objective 4, the field cooperative treatment (2.6), GLMMs were constructed for treatment and control sites separately, with time (3 survey timings, i.e., –4, 9, and 37 days after treatment) as a fixed factor and individual monitoring site as a random factor. The number of ants on individual cotton pad was used as the response variable.

3 RESULTS

3.1 Screening of active ingredients

The mean ± SD number of ants feeding on hydrogel baits across 8 replicate cups and 8 observation timings was 0.97 ± 1.1 in controls, 0.56 ± 0.89 in 0.01% thiamethoxam, 0.52 ± 0.87 in 0.01% dinotefuran, 0.66 ± 1.3 in 0.01% imidacloprid, 0.80 ± 0.80 in 0.00052% fipronil, and 0.63 ± 0.86 in 1% boric acid treatments. The effect of insecticide on the number of ants feeding was not significant for all insecticides (GLMM, $P > 0.05$). However, the number of ants feeding decreased over time and the effect of time was significant (Estimate = –0.0087, SE = 0.0032, t = –2.7, $P < 0.01$). A significant interaction between insecticide and time was detected for imidacloprid (Estimate = –0.011, SE = 0.0045, t = –2.5, $P < 0.05$). In the imidacloprid treatment, the ants became inactive and did not feed on hydrogel baits during the last 3 observations of 60, 90, and 120 min after bait provisioning. Clear symptoms of insecticide poisoning did not appear during the first 120 min of observation except for dinotefuran which paralyzed 10% of ants within 60–90 min and additional 19% within 90–120 min. However, subsequent survival curves varied significantly among different insecticides (Figure 3; $P < 0.05$ for all insecticide and control pairs). On day 7, mortality with thiamethoxam and dinotefuran was >90%, while mortality with boric acid, fipronil, and imidacloprid did not exceed 50%. In tests assessing the efficacy of thiamethoxam and dinotefuran across a range of concentrations, survival curves varied significantly among different concentrations of insecticides except for 0.001% thiamethoxam vs. 0.01% dinotefuran ($P = 0.88$) and 0.0001% dinotefuran vs. control ($P = 0.059$) (Figure 4). Mortality with 0.01% thiamethoxam reached 100% and mortality with 0.001% thiamethoxam and 0.01% dinotefuran reached 90% in 7 days. In other treatments, mortality did not exceed 40% in 7 days.

3.2 Evaluation of insecticide horizontal transfer

The mean ± SD number of donor ants feeding on hydrogel baits across 7 replicate cups and 8 observation timings was 2.0 ± 1.4 in controls, 1.9 ± 1.2 in 0.001% thiamethoxam, and 1.3 ± 1.1 in 0.01% thiamethoxam treatments. The effect of thiamethoxam concentration on the number of ants feeding was not significant (GLMM, Estimate = –6.7, SE = 43.3, t = –0.16, $P = 0.88$). However, time had a negative effect on the number of ants feeding (Estimate = –0.0076, SE = 0.0033, t = –2.3, $P < 0.05$). Interaction between thiamethoxam concentration and time was no significant (Estimate = –0.92, SE = 0.57, t = –1.6, $P = 0.11$). Physical contact between donors and recipients was observed across all treatments, all trials, and all observations conducted every 15 min during the first 1 hour. The mean total (± SD) number of recipients grooming donors during the first hour was 3.7 ± 1.1, 4.7 ± 3.3, 3.6 ± 2.6 for the control, 0.001% thiamethoxam, and 0.01% thiamethoxam treatments, respectively. Significant difference was detected in most of the pairwise comparisons of survival curves (log-rank test, $P < 0.05$) (Figure 5). The majority of donor ants feeding on 0.01% thiamethoxam bait died within 2 days while donor ants feeding on 0.001% thiamethoxam bait died more gradually, over a period of 7 days (Figure 5). Recipient mortality in the 0.01% thiamethoxam treatment was slightly higher than recipient mortality in the control (log-rank test, $P < 0.05$). However, mortality in the 0.001% thiamethoxam treatment was not different from those in the control ($P = 0.11$). Recipients carrying dead donors (necrophoresis) were occasionally observed during mortality counts. However, trophallaxis was not observed during the entire experiment.

3.3 Field palatability of hydrogel baits

Blank hydrogels containing 30% and 10% sugar (but no insecticide) were the most attractive, followed by cotton pads containing 30% and 10% sugar water (Table 1). Ten percent w/w honey water, peanut cream, and tap water were less attractive. The effect of time on the number of *T. brunneus* feeding was not significant (GLMM, Estimate = 2.9, SE = 4.0, t = 0.73, $P = 0.47$). Additionally, the interaction between food and time was not significant ($P >$ 0.05 for all foods tested).

3.4 Field cooperative treatment

The mean ± SD total number of *T. brunneus* recorded during the pre-treatment monitoring survey was 706 ± 442 in treatment sites (n = 10) and 562 ± 653 in control (no treatment) sites (n = 5) (Figure 6). At treatment sites, the abundance of *T. brunneus* significantly changed over time (GLMM, Estimate = 46.1, SE = 9.5, t = 4.9, $P < 0.001$); it decreased by 87% at 9 days post-treatment and 32% at 37 days post-treatment (Figure 6). At control sites, *T. brunneus* abundance did not change significantly over time (GLMM, Estimate = 56.8, SE = 29.6, t = 1.9, $P = 0.12$).

Survey response sheets were received from 146 of 225 residences resulting in 65% participation rate. The majority of residents regarded the bait as either easy (49%) or relatively easy (29%) to apply (Figure 7, Q1). Additionally, the majority of residents reported that the bait was highly attractive to *T. brunneus* (Q2). Fifty percent of respondents scored attractiveness as “very good” and 40% as “good”. Regarding the speed of action, 78% of respondents stated that *T. brunneus* activity became the lowest within 1–7 days after the treatment (Q3). Regarding bait efficacy, 6% of residents reported a complete absence of ants post-treatment (100% decline in ant density relative to pre-treatment counts). Forty-three percent of residents reported 90% decline and 21% reported 80% decline (Q4). The remaining 30% of residents reported control ranging from 10–70%. However, approximately 58% of residents reported that ants were still present 1 month after the treatment (Q5). A total of 6 residents observed and reported possible non-target effects including a snail (2 respondents) and a small fly. Additionally, a dead cockroach, spider, and gecko were reported, but detailed circumstances including the number of corpses, their location and exact cause of death, are unclear. In additional comments, many residents showed appreciation and gratitude for the efficacy of the hydrogel bait treatment. Many residents were eager to use the bait regularly, but some were dissatisfied by the fact that the effect was temporary.

4 DISCUSSION

Results demonstrate that neonicotinoid insecticides (thiamethoxam and dinotefuran) are highly effective against *T. brunneus*. A previous study reported that imidacloprid is effective against a related species, *T. difficilis*.[16] However, imidacloprid was not effective against *T. brunneus* in this study suggesting that insecticide susceptibility may vary across *Technomyrmex* species. A recent study examined insecticide susceptibility across 12 species of urban pest ants and demonstrated significant variation in susceptibility across species, including closely related species belonging to the same genus.[26] Boric acid and fipronil were only marginally effective despite being commonly used for ant control at the concentrations evaluated in this study. These results suggest that *T. brunneus* is less susceptible to insecticides than common household pest ant species in Japan. In contrast to thiamethoxam, dinotefuran paralyzed *T. brunneus* relatively quickly at its effective concentration (< 90 min at 0.01%). The quick onset of symptoms with dinotefuran might prevent *T. brunneus* from mass recruitment to hydrogel baits. Therefore, we conclude that thiamethoxam is the most effective insecticide for use against *T. brunneus*.

Results of horizontal transfer tests suggest that thiamethoxam is not effectively transferred from donor to recipient ants. *Technomyrmex brunneus* do not engage in trophallaxis,[23] thus horizontal transfer of insecticides via trophallaxis cannot be expected. Our results are consistent with the hypothesis that trophallaxis is important for bait transfer and that *T. brunneus* is more difficult to control than other ant species that perform trophallaxis. However, low level mortality in recipients exposed to donors feeding on thiamethoxam bait suggests that weak horizontal transfer might be occurring through other behaviors such as mutual grooming which was frequently observed among *T. brunneus*. Various behavioral mechanisms may have contributed to the transfer of thiamethoxam including direct contact, mutual grooming, and possibly accidental ingestion of small particles of baits while grooming. Necrophoresis, where live ants carry the corpses of dead ants, may have also played a role and previous studies demonstrate that necrophoresis plays a significant role in horizontal insecticide transfer.[27, 28] Additionally, a colony-level experiment including queens and brood should be conducted to fully assess the potential for horizontal transfer. The presence of brood might facilitate the production of trophic eggs by workers. A portion of insecticide ingested by the workers might be transferred to trophic eggs and subsequently larvae that feed on trophic eggs.

In the absence of horizontal transfer mediated by trophallaxis, insecticidal baits may still be effective against *T. brunneus* if they attract and kill a sufficient number of *T. brunneus*. This is because mass mortality of workers can lead to colony starvation, which in turn can reduce larval development and the production of reproductives. In the current study, thiamethoxam formulated at ≤ 0.01% did not diminish the palatability of hydrogel baits, which is in agreement with previous studies which utilized hydrogel baits containing thiamethoxam for controlling invasive ants.[4, 5]

Pest ants may reject commercial baits in favor of hemipteran honeydew and other competing food sources.[21, 29] Our field tests demonstrated that hydrogels containing 10% or 30% w/w sugar are more attractive to *T. brunneus* than sugar water of equivalent concentration. In a previous study, sugar water on cotton pads was more attractive to *T. brunneus* than commercial ant baits available in Japan.[20] That hydrogels are more attractive than sugar water suggests that hydrogel baits may effectively compete with natural carbohydrate-rich liquid food sources such as honeydew and nectar. Results of the current study demonstrate that hydrogel baits containing low concentration thiamethoxam were nonrepellent, the toxicant action was delayed and that the sucrose solution used was highly palatable. All of these features are highly desirable for a bait that will be effective at controlling *T. brunneus*. As such, hydrogel baits may offer a low-cost and low-maintenance tool for managing *T. brunneus*.

Previous studies primarily used hydrogel baits containing 25% sucrose for controlling Argentine ants,[5, 9, 11] and Cabrera et al. (2021) showed that 20% and 30% sucrose are more suitable for liquid uptake from hydrogels by Argentine ants than 40% and 50% sucrose.[30] Warner and Scheffrahn (2005) reported that *T. difficilis* prefers 25%–40% sucrose water.[16] In the present study, the concentration of sugar (10% or 30%) did not significantly affect the palatability of hydrogels or sugar water and therefore there appears to be flexibility in preparing baits that maximize efficacy within different environmental conditions. For example, Putri et al. (2021) suggested that the *T. brunneus* population on Hachijo Island is at a lower trophic level than populations in other invaded areas and that the Hachijo population relies heavily on hemipteran honeydew.[31] Thus, a high concentration of sugar in hydrogel bait may make the bait highly attractive to *T. brunneus*, especially during summer when the densities of honeydew-producing hemipterans may increase.

Results of the community-wide treatment demonstrate that hydrogel bait containing 30% sucrose as attractant and 0.001% thiamethoxam as toxicant is highly effective against *T. brunneus*. A significant reduction of *T. brunneus* was achieved around the treated structures and the population was reduced by an average of 87 ± 7% within 9 days (range: 78–98%). This suggests that insecticidal baits can suppress *T. brunneus* populations even in the absence of horizontal transfer via trophallaxis. However, the reduction was temporary and ant counts recovered to an average of 68 ± 47% (range: 4–158%) of pre-treatment counts approximately 1 month post-treatment. *Technomyrmex brunneus* workers require >1 month to develop from egg to adult.[23] Thus, without sufficient workers which take care of brood, it may be difficult for *T. brunneus* colonies to fully recover in 1 month, even if queens were not killed by hydrogel baits. The observed recovery suggests re-invasion of treated areas by ants from nearby untreated areas. Residential housing on Hachijo Island is relatively sparse and in close proximity to forested areas (Figure 2). It is likely that the untreated green areas surrounding residential housing served as the source of re-infestation. The re-invasion may have been accelerated by the propensity of *T. brunneus* in the Hachijo population to form supercolonies, a feature that appears unique to the Hajicho population and has not yet been reported in other populations.[19, 31]

Results of the survey were consistent and Hachijo residents recognized that the hydrogel bait is relatively easy to apply, highly attractive, relatively fast-acting and highly effective against *T. brunneus*. In the future, regular distribution of hydrogel baits to residents and follow-up treatments may effectively suppress *T. brunneus* density in the residential area.

With regard to non-target effects of hydrogel baits, most Hachijo residents did not observe any negative effects. Neonicotinoid insecticides can have negative impacts on non-target insects, especially pollinators and aquatic insects.[13,14] Previous studies demonstrated that hydrogel baits pose minimal risks to pollinators including honeybees, because they are not attracted to hydrogel baits.[32, 33] Hachijo Island harbors many endemic species and subspecies, and ground crawling insects such as *Prosopocoilus hachijoensis* and *Dorcus titanus hachijoensis* (both Coleoptera: Lucanidae) which may encounter and feed on hydrogel baits.[18] Furthermore, some residents reported possible effects, which are worth paying attention to. Therefore, the potential impact of hydrogel baits on non-target organisms should be carefully examined in future research.

In conclusion, hydrogel baits containing thiamethoxam as the active ingredient are palatable and effective against invasive populations of *T. brunneus*. Lack of trophallaxis limits the horizontal transfer of thiamethoxam within *T. brunneus* colonies and may make the eradication difficult. However, a community-wide hydrogel bait treatment for *T. brunneus* was highly effective and reduced *T. brunneus* densities throughout the treatment area. Our results demonstrate that a large network of highly motivated and properly trained members of the public can be highly effective in managing invasive ant populations in urban environments using hydrogel baits.

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Table 1. Mean ± SD number of *Technomyrmex brunneus* feeding from hydrogels and other food matrices at two times in the field palatability test (n = 6). Food sources with the same superscript letter are not statistically different by Tukey's multiple comparison test in the GLMM analysis ($P > 0.05$).

Food source	Number of *T. brunneus* feeding (mean ± SD)	
	1 hour	3 hours
Hydrogel bait (30% sugar) ^{a}	63 ± 31	54 ± 21
Hydrogel bait (10% sugar) ^{ab}	54 ± 23	62 ± 19
30% sugar water ^{bc}	24 ± 21	33 ± 18
10% sugar water ^{bc}	26 ± 14	28 ± 15
10% honey water bait ^{c}	10 ± 12	19 ± 13
Peanut cream ^{c}	0 ± 0	2 ± 3
Tap water ^{c}	2 ± 2	8 ± 5

Figure 1. Trunk trails of *Technomyrmex brunneus* surrounding a building and entering through a gap around the door. The arrow indicates a hydrogel bait.

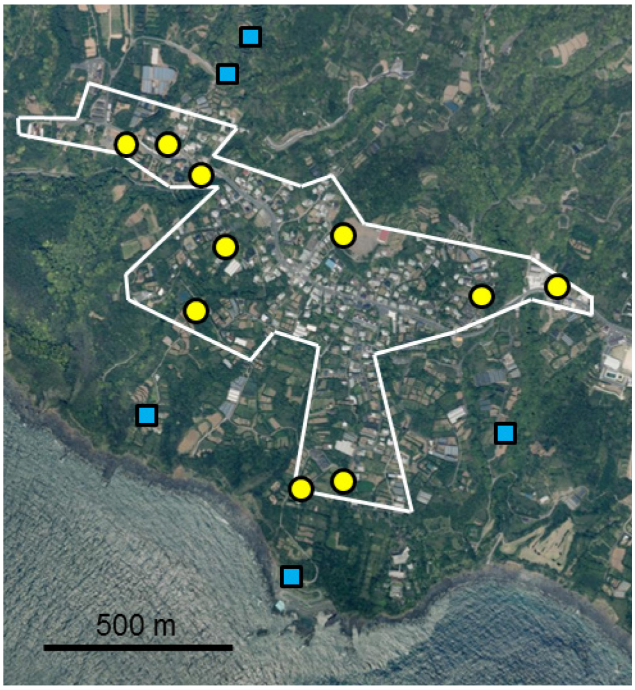

Figure 2. A map of Kashitate Village, Hachijo Island. The area within the white line indicates the residential area treated during the community-wide *Technomyrmex brunneus* management program. Circles indicate treated sites and squares control (untreated) sites used for ant density monitoring. The map was created by editing the vector tiles published by the Geospatial Information Authority of Japan (https://maps.gsi.go.jp/vector/).

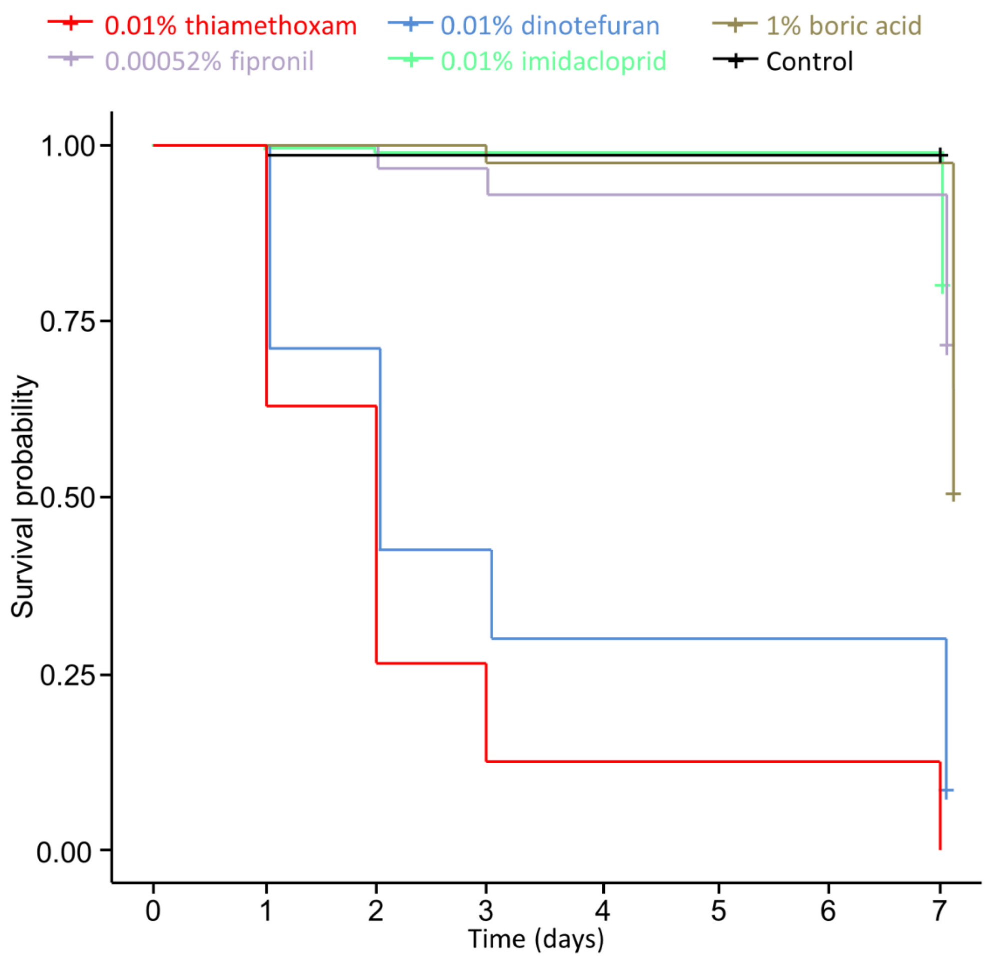

Figure 3. Kaplan-Meier survival curves for *Technomyrmex brunneus* fed with hydrogel baits containing various candidate insecticides and 30% w/w sugar (n = 160).

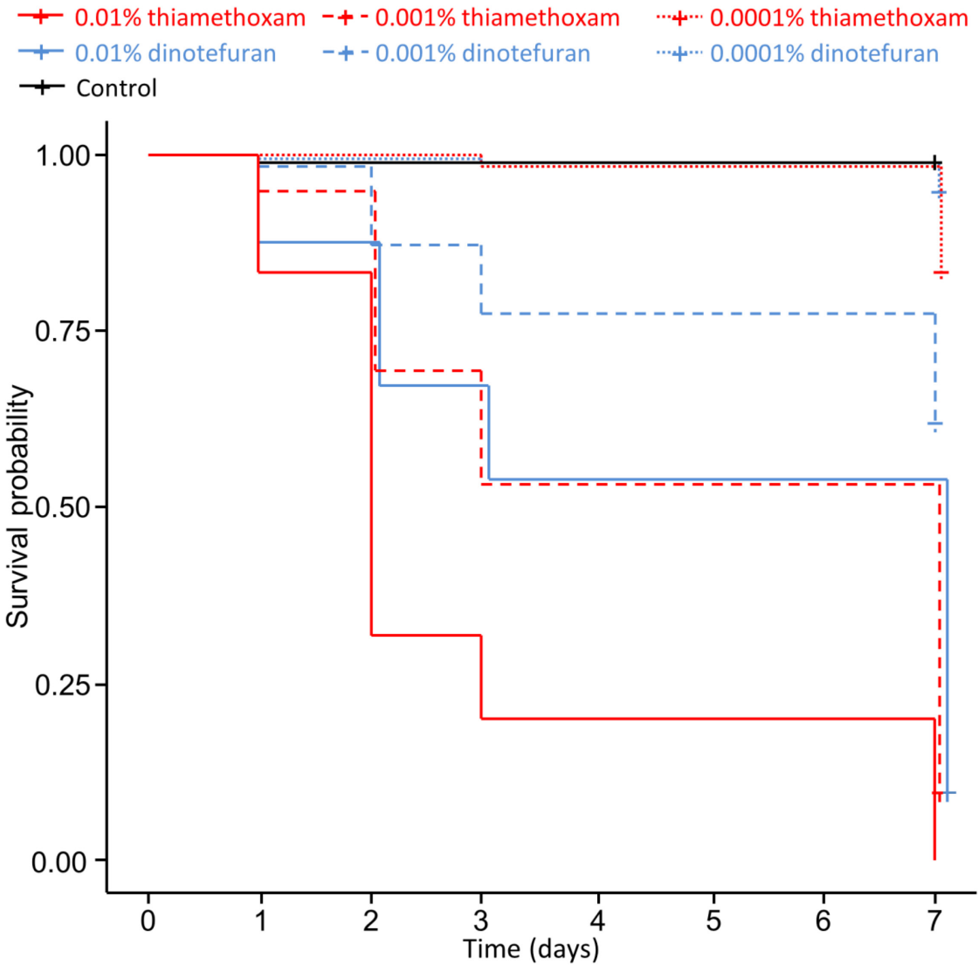

Figure 4. Kaplan-Meier survival curves for *Technomyrmex brunneus* fed with hydrogel baits containing different concentrations of thiamethoxam or dinotefuran and 30% w/w sugar (n = 160).

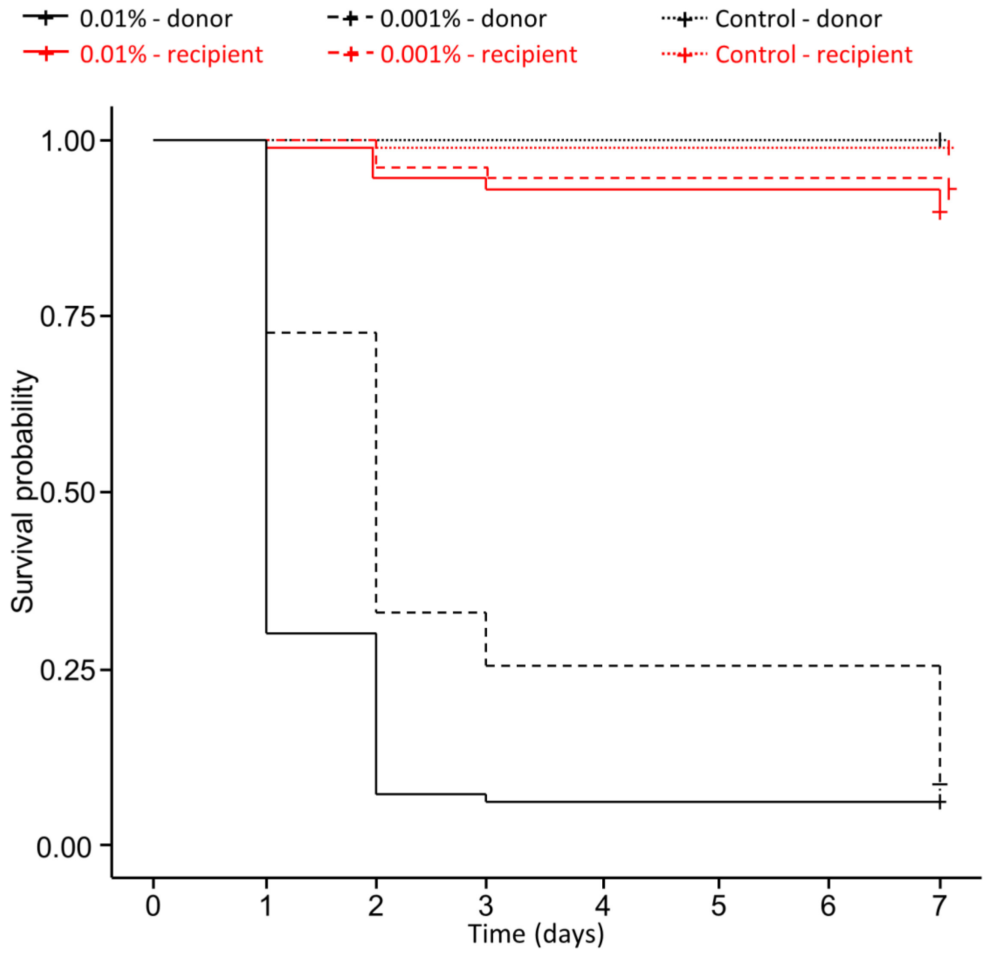

Figure 5. Kaplan-Meier survival curves for *Technomyrmex brunneus* donors fed with hydrogel baits containing 0, 0.001, or 0.01% thiamethoxam and 30% w/w sugar and recipients cohabited with the donors (n = 70).

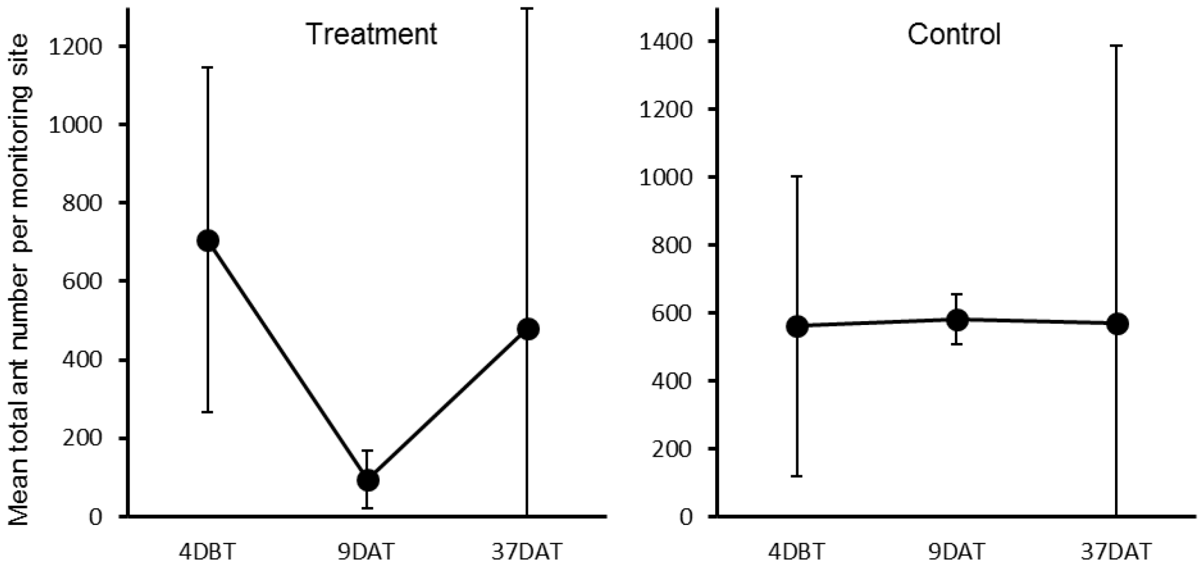

Figure 6. Mean ± SD total number of *Technomyrmex brunneus* at the monitoring sites of the area-wide management program in Hachijo Island (n = 10 for treatment and 5 for control). Monitoring surveys were conducted at 4 days before treatment (DBT) and 9 and 37 days after treatment (DAT).

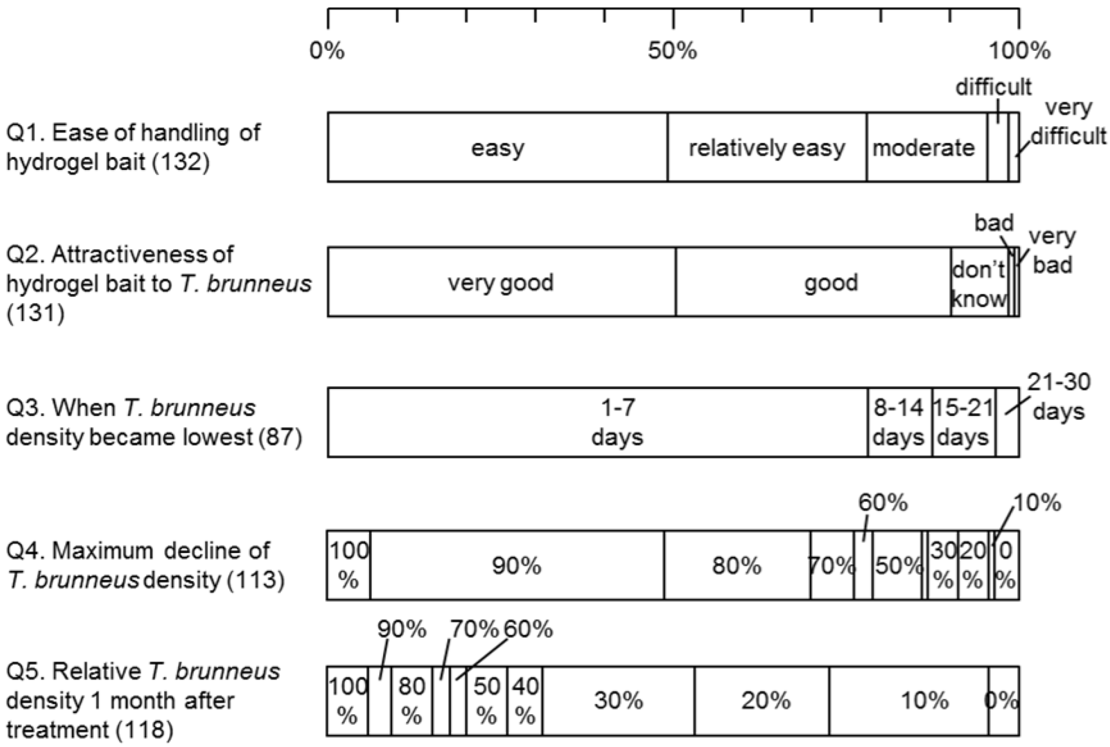

Figure 7. Survey results showing the percentage of responses for each question. The total number of responses to each question is indicated in parentheses after the question caption.