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RESEARCH ARTICLE - ANTS

Development of New Boric Acid Gel Baits for Use on Invasive Ants (Hymenoptera: Formicidae)

LN Kafle, AC Neupane, YM Wang, SR Gangai

Department of Tropical Agriculture and International Cooperation, National Pingtung University of Science and Technology, Pingtung, Taiwan

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Corresponding author
Samantha Rose Gangai
Department of Tropical Agriculture and International Cooperation
National Pingtung University of Science and Technology
1, Sheufu Rd, Neipu, Pingtung 91201, Taiwan.
E-Mail: chamandah@gmail.com

Abstract

The current control measures used against common household ants in urban and agricultural settings include perimeter insecticide applications. These often have the potential to cause problems of poisoning non-target organisms, due to the insecticidal runoff and environmental contamination. A gel-baiting technique is the most effective tool to control ants with less insecticides released into the environment. In this study two commercial gel baits; the Boric acid (2.5% boric acid) and the Fipronil (0.01% fipronil) baits, were evaluated against laboratory made baits (lab baits). The lab baits, consisted of: 1.5% boric + fructose (F1.5), 1.5% boric acid + fructose + molasses (M1.5), 3% boric acid + fructose (F3), and 3% boric + fructose + molasses (M3) were evaluated based on preference and mortality rates of the common household ant species: the Asian needle ant, *Monomorium chinensis,* Santschi, and the Yellow crazy ant, *Anoplolepis gracilipes,* Smith, under laboratory conditions. An inconsistent preference was observed between species and different baits; however, the fipronil bait and the lab bait M3, were preferred more by both ant species compared to the other baits tested. Lab bait M3 also had a faster killing speed than the boric acid bait and the lab bait F3.Based on the results it was concluded that lab bait M3 was a more efficient ant bait and is a potential alternative control measure to the current commercial baits.

Introduction

Ants comprise 5% of the world's one hundred worst invasive alien species and of the seventeen land invertebrates listed, five (28%) are ants (Vander Meer & Milne, 2017). Among the most problematic invasive species, the Asian needle ant, *Monomorium chinensis,* Santschi and the Yellow crazy ant, *Anoplolepis gracilipes*, Smith, cause devastating environmental and urban problems all over the world and area threat to local biodiversity (Zheng et al., 2008). Current control measures of these species include perimeter spraying or direct application to nest sites for successful control. However, this can result in insecticidal run-off and environmental contamination often poisons other non-target organisms (Welzel & Choe, 2016). Kafle et al. (2010) stated that the solid bait design was not ideal due to a tendency to become excessively hydrated or dehydrated and needing frequent maintenance. Considering that most baits contain toxic substances, they are therefore environmentally hazardous and arising concern to world wide health agencies and environment authorities (Kafle & Shih, 2013).

Fipronil is a broad use insecticide used to control ants and other insect pests. It is highly toxic to sea and freshwater vertebrates and invertebrates. Furthermore, Fipronil has been found to be highly toxic to some birds, although non-toxic to ducks. It has also been found as highly toxic to honey bees, but non-toxic to earthworms (Jackson et al., 2009). Fibronil is frequently found in urban waterways and aquatic systems in amounts exceeding the LC_{50} values and therefore there is concern for its potential impact on non-target organisms, as has been reported in California (Welzel & Choe, 2016).

Similarly, Boric acid is a pesticide that can also be found in nature and has been used for ant control since the early 1900's. Boric acid is slow acting and non-repellent

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thereby enhancing long-term ingestion (Klotz & Moss, 1996: Klotz & Williams, 1996). Boric acid and sodium salts can be used to control insects, spiders, mites, algae, moulds, fungi, and weeds. The delayed activity of boric acid promotes a thorough distribution of the active ingredient within the nest, leading to the death of the entire colony (Klotz & Williams, 1996). Commercial ant baits with boric acid as an active ingredient typically use concentrations of > 5% (Klotz et al., 2000).

During this study, we formulated a new composition of ant bait with boric acid as an active ingredient (AI). In order to determine an effective range of boric acid, we compared different lab baits with two concentrations of boric acid (1.5% and 3%) against two commercial ant baits; being them fipronil (0.014%) and boric acid (2.5%). The study focused on (1) evaluating the feeding preferences for lab baits against commercial baits, and (2) comparing the efficacy of the most preferred lab bait against commercial baits. The results of this study aim to develop new gel baits with a specific percentage of boric acid as the active ingredient (AI) for effective control of common household ants; *M. chinensis* and *A. gracilipes*.

Materials and Methods

The source of ants

Polygyne colonies of the Asian needle ant, *Monomorium chinensis*, Santschi and the Yellow crazy ant, *Anoplolepis gracilipes*, Smith, were obtained by excavation from field populations located around the National Pingtung University of Science and Technology campus, Pingtung, Taiwan. The ants were separated from the soil using the water drip method, as described by Kafle et al. (2008, 2009), and Chen (2007), and reared under ambient laboratory conditions (27±1 °C and 50±3% RH), photoperiod of 14:10 h (L:D) and placed on a standard diet of water, meal worm larvae, peanut butter, and 10% sugar water at least one week before the tests. The test population, usually made up of the major workers, were isolated from the main population and starved for 24 hours before each test was conducted.

Preparation of the laboratory baits

A stock solution made up of 25% boric acid was prepared by heating25g of boric acid powder (99%) (Shimada Chemical Works, Taipei, Taiwan) and75gof fructose (SunRight Foods Corporation, Taipei, Taiwan) at a temperature of 140ºC for 15 minutes, in order to completely dissolve the boric acid in the fructose. After cooling the mixture, 6g of the stock solution was added to 19g of either fructose or molasses to make up1.5% boric baits. Similarly, to make up 3% boric baits, 13g of fructose or molasses was added to 12g of stock solution for 25g in each lab bait. The specific ratios of each component contained in the lab baits are shown in Table 1.

Table 1. Ratio of each constituent used in new laboratory bait preparations.

Constituent (%)	Lab baits*			
	F1.5	F3	M1.5	M3
Fructose	94	88	0	0
Molasses	0	0	94	88
Stock solution (25% Boric acid)	6	12	6	12
Total vol. (%)	100	100	100	100

*F = Fructose, M = Molasses, 1.5 = 1.5% boric acid and 3 = 3% boric acid

Commercial Baits

Two popular commercial ant baits used in Taiwan were compared for efficacy against the laboratory baits: the boric acid bait (2.5% boric acid, Chung Tai Sing Chemical Industry Company Limited, Hsinchu, Taiwan) and the fipronil bait (0.01% fipronil, Family Consumer Products Company Limited, Miaoli, Taiwan). The baits were bought at the local market.

Preference Tests

In order to evaluate the feeding preferencesof the *M. chinensis* and *A. gracilipes* on the four laboratory baits (Table 1) and two commercial baits, similar studies undertaken by Kafle et al. (2010) and Klotz (2000) were adapted for this test. Acrylic rectangular foraging stations (18.5cm x 10.5cm x 4cm (L x W x H) were set up and modified to suit the purpose of the experiment. Fluon was applied to the inner vertical surfaces to prevent ants from escaping the container. The test baits were arranged side by side on parafilm squares (1 cm x 1 cm) along one end of the setup and water was not provided during the test period. An artificial cardboard nest (2 cm x 2 cm) setup, as an artificial ant nest, were placed 1cm away from the inner wall and10cm away from the baits.

The feeding preferences were recorded on 60-minute videos. These observations were reviewed and interval counts were done at 5 minute frames. Ant-to-bait counts were determined by the number of ants actually feeding on the baits or foraging on the individual parafilm squares for at least 10 seconds. During the test period any dead ant was removed and replaced with live ants from the isolated population. This was to maintain the test population during the study period.

A hundred *M. chinensis* and fifty *A. gracilipes* workers from the test populations were used in each individual test. Each test was replicated four times under the laboratory conditions at temperatures of 25 ± 3ºC and RH 52 ± 3% and 14:10h L:D photoperiods.

Mortality Tests

According to the results of the preference tests, the lab baits F3 and M3 had the highest preferences used in the mortality test. Rectangular foraging stations similar to those used in the preference tests were set as described by Klotz et al. (2000) and Kafle et al. (2010), and modified for this experiment.

Fluon was applied to the inner vertical surfaces to prevent ants from escaping. A single test bait per feeding arena was placed at one end of the foraging arena on a parafilm strip 1cm away from the vertical wall. An artificial cardboard nest was placed 10cm away from the bait at the opposite end of the foraging arena 1cm away from the inner wall. Water was provided during the mortality tests and mealworm larvae were provided as food in the control sets and in all mortality tests.

Efficacies were compared across the baits; the boric acid, fipronil, lab bait F3, and lab bait M3, in tests similar to those described by Kafle et al. (2010). Observations of the test were recorded at 3-hour intervals for the first 24 hours and every 6 hours thereafter, until a hundred percent mortality was reached. Thirty worker ants from the test populations of both species were used and each test was replicated four times under laboratory conditions;at temperatures of 25 ± 3ºC and RH 52 ± 3% and 14:10h L:D photoperiods.

Data collection and analysis

Each test setup was randomized and the data was sorted and compared on an Excel spreadsheet using the procedures described by Vander Meer (2017) and Kafle et al. (2010). The lethal time (LT_{50}) was calculated through probit analysis using StatPlus (2017) and the means were compared using SNK of SAS (2017).

Results

Preference Tests

Two fructose based lab baits, the F1.5 and F3 (Table 1), were compared with the two commercial baits, fipronil and boric acid, to determine the preference of *M. chinensis* and *A. gracilipes*. The percentage of *M. chinensis* observed foraging on the Fipronil bait was significantly higher than those observed on the rest of tested baits; however, the number of ants observed on the lab bait F1.5, the boric acid bait and the lab bait F3 were not significantly different ($F = 6.2$, $p < 0.01$) (Table 2).

The number of *A. gracilipes* observed foraging on the lab bait F3 was significantly higher than the other baits tested.

Table 2. Preference of *Monomorium chinensis* and *Anoplolepis gracilipes* on new fructose and commercial baits under laboratory conditions.

Baits tested	No. of ants foraging (Mean ± SE)*	
	Monomorium chinensis	*Anoplolepis gracilipes*
Boric acid bait (2.5% Boric acid)	8.26 ± 0.37b	12 ± 3.72a
Fipronil bait (0.01% Fipronil)	12.25 ± 1.12a	8.25 ± 1.38a
Lab bait 3F (5% Boric acid)	7.00 ± 0.93b	8.25 ± 1.11a
Lab bait 4F (10% Boric acid)	5.25 ± 0.8b	15.25 ± 3.68b

*Means within the same column followed by the same letter are not significantly different (p<0.05) (SNK test, SAS, 2017).

The boric acid bait had the next highest preference. However, the number of *A. gracilipes* observed foraging on the two commercial baits and the lab bait F1.5 were not significantly different ($F = 1.49$, $p < 0.27$) (Table 2). Similarly, the number of *M. chinensis* observed foraging on the fipronil bait was significantly higher than the other three baits. However, the number of ants observed on the lab bait M1.5 and fipronil bait or lab bait M3 and the boric acid bait were not significantly different ($F = 12.84$, $p < 0.01$) (Table 3).

The number of *M. chinensis* observed foraging on the Fipronil bait was statistically higher than the other three baits tested. However, there was no significant difference observed on the percentage of ants on all the baits tested ($F = 0.14$, $p < 0.93$) (Table 3). The preference tests showed that lab bait M3 was more preferred by *M. chinensis* than the fructose based baits, while *A. gracilipes* preferred both the lab F3 and lab M3 baits. Therefore, only the lab baits F3 and M3 were evaluated against the two commercial baits in the mortality tests.

Table 3. Preference of *Monomorium chinensis* and *Anoplolepis gracilipes* on new molasses and commercial baits under laboratory conditions.

Baits tested	No. of ants foraging (Mean ± SE)*	
	Monomorium chinensis	*Anoplolepis gracilipes*
Boric acid bait (2.5% Boric acid)	5.74 ± 0.97b	10.75 ± 4.25a
Fipronil bait (0.01% Fipronil)	13.09 ± 2.88a	13 ± 3.24a
Lab bait 3M (5% Boric acid)	7.82 ± 3.39b	9.75 ± 4.11a
Lab bait 4M (10% Boric acid)	12.19 ± 4.77a	12.25 ± 3.97a

*Means within the same column followed by the same letter are not significantly different (p<0.05) (SNK test, SAS, 2017).

Mortality Tests

Mortality of *M. chinensis* by lab baits F3 and M3 against two commercialwere compared with the two commercial baits, fipronil and boric acid under the laboratory conditions. The percentage of *M. chinensis* killed by the lab baits(F3 and M3) fipronil and boric acid baits were not significantly different at 6 HAT and 12 HAT (6 HAT: $F= 1.07$, $p < 0.41$; 12 HAT: $F = 2.61$, $p < 0.08$) (Table 4).

At 24 HAT, the percentage of ants killed by the fipronil bait was significantly higher than the lab bait M3 and the control. However, there was no significant difference in the percentage of ants killed by the boric acid, fipronil baits and the M3 or the boric acid bait, and the lab baits F3 and M3 ($F = 7.81$, $p < 0.01$) (Table 4).

The mortality rate of the *M. chinensis* by the lab bait M3, fipronil and boric acid baits were significantly higher than the control at 48, 72 and 84 HAT. However, the percentage of ants killed by all three baits was not significantly different from each other. It was observed that all baits could kill up to 100% of *M. chinensis* within 84 HAT. At 72 HAT, the lab bait F3 killed 100% ants (48 HAT: $F = 8.83$, $p < 0.01$; 72 HAT: F =

194.62, $p < 0.01$; 84 HAT: $F = 2255.53$, $p < 0.01$). Based on the LT_{50} results, fipronil was the fastest killing bait, followed by the boric acid bait the lab bait M3 and then the lab bait F3 (Table 4).

Mortality of *A. gracilipes* by lab baits F3 and M3 against two commercial were compared with the two commercial baits, fipronil and boric acid under the laboratory conditions. The percentage of *A. gracilipes* killed by the fipronil bait was significantly higher than the F3, M3 and boric acid baits at 6 HAT and 12 HAT. However, the percentage of ants killed by the boric acid bait and the lab baits F3 and M3 were not significantly different (6 HAT: $F = 136.08$, $p < 0.01$; 12 HAT: $F = 29.76$, $p < 0.01$) (Table 5).

Table 4. Mortality of *M. chinensis* by lab baits F3 and M3 against two commercial baits under laboratory conditions.

Observation time$^{\alpha}$	Ant mortality (%) (Mean ± SE)*				
	Boric acid bait	Fipronil bait	Lab bait F3	Lab bait M3	Control
6 HAT	2.62±1.02a	5.96±2.57a	0±0a	3.33±3.33a	2.50±1.60a
12 HAT	12.82±4.40a	26.94±5.51a	0.81±0.81a	25.61±15.48a	2.50±1.60a
24 HAT	46.38±11.86ab	73.86±9.96a	5.63±2.63b	44.96±17.25ab	6.67±1.36b
48 HAT	68.11±13.19a	86.10±7.60a	59.73±13.58a	62.83±13.02a	20.84±2.50b
72 HAT	93.58±4.05a	96.75±1.90a	100±0a	97.80±2.21a	24.17±1.60b
84 HAT	100±0a	100±0a	100±0a	100±0a	24.17±1.60b
LT_{50}^{β} (h)	27.67	18.23	39.01	27.92	-

*Means within the same row followed by the same letter (lower case) are not significantly different ($p < 0.05$) (SNK test, SAS, 2017)
α HAT = Hours after treatment
β LT50 values (h) were determined by Probit analysis (StatPlus, 2017)
F3 = 3% boric acid
M3 = 3% boric acid

At 24 HAT, the percentage of ants killed by the fipronil bait was significantly higher than the other three baits tested. However, the percentage of ants killed by the boric acid, fipronil and the lab bait F3 were not significant different ($F = 600.52$, $p < 0.01$) (Table 5). Similarly, at 48 HAT, the percentage of *A. gracilipes* killed by the fipronil and boric acid baits reached 100% and was significantly higher than lab baits F3 and M3. However, the percentage of ants killed by the boric acid and fipronil baits and lab bait M3 was not significantly different ($F = 296.33$, $p < 0.01$) (Table 5).

Table 5. Mortality of *A. gracilipes* by lab baits F3and M3 against two commercial baits under laboratory conditions.

Observation time$^{\alpha}$	Ant mortality (%) (Mean ± SE)*				
	Boric acid bait	Fipronil bait	Lab bait F3	Lab bait M3	Control
6 HAT	3.70±0.80c	78.60±5.57a	1.62±0.93c	14.93±1.59b	6.67±1.36bc
12 HAT	13.17±1.95c	90.32±4.62a	1.62±0.93c	43.56±13.87b	10.83±1.56c
24 HAT	98.49±1.51a	100±0a	1.62±0.93c	97.14±2.86a	20.83±2.85b
48 HAT	100±0a	100±0a	76.15±3.50b	98.57±1.43a	25.83±3.44c
72 HAT	100.0±0a	100.0±0a	100.0±0a	100.0±0a	26.67±3.04b
LT_{50}^{β}	16.63	3.49	36.25	12.44	-

* Means within the same row followed by the same letter (lower case) are not significantly different ($p < 0.05$) (SNK test, SAS, 2017)
α HAT = Hours after treatment
β LT50 values (h) were determined by probit analysis (StatPlus, 2017)
F3 = 3% boric acid
M3 = 3% boric acid

The percentage of *A. gracilipes* killed by the lab baits F3 and M3, and fipronil and boric acid baits reached 100% of ants at 72 HAT and the percentage of ants killed by all tested baits was significantly higher than the control ($F = 580.94$, $p < 0.01$). Based on the LT_{50} value the fipronil bait was the fastest killing bait of *M. chinensis* and *A. gracilipes*, followed by the lab bait M3, the boric acid bait and the lab bait F3 (Table 5).

Discussion

Molasses and fructose are two of the most popular natural sugars used in insect control. A similar study undertaken by Ulloa-Chacon and Jaramillo (2003) emphasized that toxic baits prepared with insecticides added to a sugar solution were attractive to the Ghost ant and appropriate for use when implemented in trials for the control of various species, similar to the Argentine ant (*Iridomyrmex humilis* Mayr).

During the preference tests on the molasses baits, a higher percentage of ants were observed to be actively foraging on the lab baits M1.5 and M3. The ants were attracted to the molasses over the fructose mainly due to the sucrose content, and thus both the *M. chinensis* and *A. gracilipes* were lured when it was used in these baits (Binkley & Wolfrom, 1953; Curtin, 1983; Saric et al., 2016).

Both *M. chinensis* and *A. gracilipes* are generalist feeders that seek out sources of carbohydrates, lipids and proteins as food sources (Vanderwoude et al., 2006). Therefore, it was observed how both species in this study actively fed on the sugar based baits. They were able to exploit their food resources by communicating with odour trails and rapid recruitment (Sparks, 2015) in a similar way. They also may have been able to communicate danger when workers were stuck in the baits. During the study, it was observed that when workers became stuck in the bait and were left there for a while, other ants also ceased feeding. This behavior was observed in both *M. chinensis* and *A. gracilipes*.

Bluthgen and Fiedler (2004a) observed the preference for sugars and amino acids of the Nectarivorous ants in an Australian tropical rain forest. Using artificial nectar solutions, the feeding behavior of fifty-one ant species on these solutions was recorded. The results stated that preferences among carbohydrates were principally consistent between ant species. A very significant observation was that many of the ant species preferred: sugar, glucose, fructose or sucrose solutions containing mixtures of amino acids, such as: alanine; arginine, asparagine, cysteine, glutamic acid, glycine, histidine, isoleucine, leucine, lysine, methionine, phenylalanine, proline, serine, threonine, tyrosine, valine, and other substances in natural nectar over pure sugar solutions. These results correspond with previous studies thus confirming the variability in amino acids and carbohydrates proposes to play a key role in ant preferences and nutrition (Bluthgen & Fiedler, 2004b; Bluthgen et al., 2004;Vander Meer et al., 1995; Lanza, 1993).

In a study on the bait preferences and toxicity of insecticides to White-footed ants, *Technomyrmex albipes*, Warner (2003) noted that fructose was found to be more significant than the nectar of *Brownea* sp., or other sugars, while both the nectar and fructose of *Clerodendrum myricoides* were significantly favored more than glucose and sucrose. This is evidence that different species of ants have a specific preference for sugar types. Therefore, the results varied in the toxicity tests.

All baits achieved 100% mortality by 72 HAT. The killing speed of lab bait M3 was faster than the boric acid bait and the lab bait F3. A main reason for this may be the ingredients used in the baits. The lab bait F3 contained just fructose and boric acid. However, lab bait M3 contained fructose, molasses and boric acid, which may have had an impact on the feeding preference and thus the results.

The killing speed of lab baits F3 and M3 against *M. chinensis* and *A. gracilipes* were faster than the boric acid bait and slower than the fipronil bait. It is assumed this is due to the different active ingredients used in each of the baits. The fipronil bait used in this study contained 0.01% active ingredient. Fipronil is a sweet smelling poison that also aided it as a preference. It is toxic to insects by contact or ingestion (Jackson et al., 2009). Thus, is used in many products when a delayed kill is desired. Fipronil disrupts the insect central nervous system and causes the hyperexcitation of nerves and muscles resulting in death.

Klotz et al. (2000) presumes that although there is little information available concerning the physiological mode of action of boric acid on insects, it has been shown that borate ions form strong complexes with sugar alcohols, and other organic functional groups. Harper et al. (2012) also suggest that boron may be involved in the disruption of intercellular adhesion because saturated boric acid solutions can be used to dissociate cells. Some advantages of using boric acid as an active ingredient in ant baits are the delayed activity and solubility in water. At a low concentration, boric acid is slow-acting and less likely to be repellent (Klotz & Williams, 1996).

As federal and state laws become more stringent on the use of residual insecticide spray, these baiting techniques may provide an effective control strategy (Welzel & Choe, 2016). The test formulations proved that the boric acid based molasses bait (M3) was an efficient formula. The results of this study showed, i) the new Lab bait M3 is a potential alternative to current commercial gel baits, and ii) boric acid can be used as an alternative for the hazardous pesticide like fipronil, as the active ingredient in ant gel baits.

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Author's contribution

The study concept and design: L. Kafle and Y. M. Wang, the acquisition of data: SR Gangai, Analysis and Interpretation of data: A.C. Neupane, S.R Gangai and L. Kafle, Manuscript preparation: A.C. Neupane and L. Kafle, and Critical revision: L. Kafle, A. C. Neupane and Y. M. Wang.

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