PARTICLE SIZE AND BAIT PREFERENCE OF THE RED IMPORTED FIRE ANT, *SOLENOPSIS INVICTA* BUREN (HYMENOPTERA: FORMICIDAE)

A Thesis

by

RICHARD RYAN NEFF

Submitted to the Office of Graduate Studies of
Texas A&M University
in partial fulfillment of the requirements for the degree of

MASTER OF SCIENCE

August 2010

Major Subject: Entomology

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Approved by:

Chair of Committee,	Roger E. Gold
Committee Members,	Bastiaan Drees
	Leon H. Russell
	Spencer Behmer
Head of Department,	Kevin Heinz

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ABSTRACT

Particle Size and Bait Preference of the Red Imported Fire Ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae). (August 2010)

Richard Ryan Neff, B.S., Texas A&M University

Chair of Advisory Committee: Dr. Roger E. Gold

One of the most popular methods for achieving control of the Red Imported Fire Ant, *Solenopsis invicta* Buren, is through the use of broadcast baits. Several factors contribute to bait efficacy, one of which may be particle size. The goals of these laboratory studies were to determine particle size and bait preference using Advance® Select Granular Ant Bait and Advance® Carpenter Ant Scatter Bait, determine the effect of starvation on bait removal and recruitment to Carpenter Ant Scatter Bait, and determine if any correlation existed between head capsule width and particle size selected.

Experimental colonies removed significantly more 1400-2000 µm particles of Select Granular Ant Bait, while ants foraging on Carpenter Ant Scatter Bait preferred 1000-1400 µm particles. Mean number of ants present at bait mirrored results from bait removal test. Ants displayed a preference for Carpenter Ant Scatter Bait based on mean number of ants present at bait for the 10-d foraging period.

For starvation assays, significant differences in bait removal and number of ants present occurred in the 0-d group. Ants starved for 5 d removed significantly more bait

of all particle sizes, and removed greater amounts of 1000-1400 µm Carpenter Ant Scatter Bait than other sizes.

Head width reliably predicted particle size selected, but the linear model explained very little of the observed variation for ants foraging on Select Granular Ant Bait ($R^2 = 0.043$) or Carpenter Ant Scatter Bait ($R^2 = 0.047$). This study supported the significant role of bait size and starvation period in *S. invicta* bait preference, and demonstrated how size preference may vary depending on bait type.

ACKNOWLEDGEMENTS

First, I would like to thank Dr. Roger E. Gold, my graduate committee chairman. Had it not been for Dr. Gold, I would not have entered the field of entomology. In addition, he provided me an opportunity not only to attend graduate school, but to earn a salary doing something I thoroughly enjoy, teaching. He makes certain we are well provided for, and is always available if I have questions. Thank you also to Dr. Robert T. Puckett, the Assistant Research Scientist at the Center for Urban and Structural Entomology. He has, more so than anyone else, critically discussed my research objectives, methods, and conclusions. His knowledge of statistics has been of great help, and his suggestions regarding controls virtually saved my thesis.

Secondly, I would like to thank Dr. Baastian 'Bart' Drees, a committee member who contributed substantially to my understanding of experimental design. I would also like to thank Dr. Leon Russell and Dr. Spencer Behmer for their support as part of my committee. I would also like to thank Bill Summerlin, who showed me the ropes of working with fire ant colonies, as well as the finer points of maintaining laboratory colonies. Bill is one of the nicest and most underrated scientists I have met in my very brief entomological career.

A special thanks to Dr. Alejandro Calixto for his help with statistical considerations.

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INTRODUCTION

The genus Solenopsis, to which fire ants belong, contains reoughly 185 described species and belongs to the largest and most diverse ant subfamily, the Myrmicinae (Tschinkel 2006; Pitts et al. 2005). Pitts et al. (2005) further revised *Solenopsis* into four distinct species groups: the *S. virulens* species group, the *S. tridens* species group, the *S. geminata* species group (native fire ants), and the *S. saevissima* species group (imported fire ants). While most *Solenopsis* species are lestobiotic and have small, monomorphic workers, 20 species differ significantly in terms of biology and social organization. These species are characterized by polymorphic workers, large colonies, and aggressive defensive habits (Pitts et al. 2005). Included among these 20 species is the Red Imported Fire Ant (RIFA), *Solenopsis invicta* Buren.

Solenopsis invicta is an invasive ant species native to South America, most likely originating in the Mesopotamia flood plain near Formosa, Argentina (Tschinkel 2006; Caldera et al. 2008). Since it arrived in Mobile, Alabama roughly 75 years ago (between 1933-1945), *S. invicta* has spread across the southern United States, from California to the Carolinas, and is widely distributed across the Texas-Mexico border (Tschinkel 2006; Lofgren et al. 1975; Sanchez-Pena et al. 2005). As of 2000, *S. invicta* had infested over 56 million acres in Texas, with 160 counties under quarantine (Lard et al. 2002). *S. invicta* disperse via mating flights or budding, depending on social form (discussed below), but the significant spread observed in the 1940's and 1950's was due in large part to transport of infested nursery stock (Lofgren et al. 1975).

This thesis follows the style of the Journal of Economic Entomology.

Solenopsis invicta exists in two genetically distinct social forms; monogyne and polygyne. Monogyne colonies consist of a single queen and her offspring. The colonies are territorial and, in Texas, average ~295 mounds per ha (Tschinkel 2006). New monogyne colonies are established when inseminated queens land post nuptial flight, find or dig a nest, and survive the claustral and incipient phases of colony growth. In contrast, polygyne colonies contain multiple queens, with as many as 700 queens per mound (Tschinkel 2006). Polygyne workers tolerate non-nestmate workers, effectively eliminating territoriality, and can occur at densities of 680 mounds per ha (Tschinkel 2006). These higher population densities increase the ecological and human impact where this social form is found (King and Tschinkel 2008).

Solenopsis invicta are opportunistic invaders and are especially adept at colonizing disturbed habitats, possibly because of their origin in the flood plains of South America. It is believed that *S. invicta* have proliferated in North America because they are free from the constraints placed upon them by competitors and natural enemies in their native range, a theory known as the enemy-release hypothesis (Hajek 2004). Tschinkel (2006) suggested *S. invicta* benefited from the widespread use of nonspecific pesticides like Mirex and Ferriamicide, which decimated native ant populations in addition to *S. invicta* populations. The empty habitats left by using such indiscriminate insecticides were left for the most aggressive colonizer, which was *S. invicta* in many North American habitats (Tschinkel 2006; Lofgren et al. 1975; King et al. 2009). *S. invicta* also benefit from the presence of disturbed (plowed, tilled, or grazed) pastures and fields (King et al. 2009), the building of roadways (Deyrup et al. 2000), and other human perturbations. King and Tschinkel (2008) used mowed and plowed plots vs.

undisturbed control plots to show that human activity was the driving force of negative effects on native communities, not biological invasion.

Solenopsis invicta are major agricultural and urban pests throughout much of the southeastern United States. Lard et al. (2001) estimated the annual economic impact of *S. invicta* on the households, schools, cities, and golf courses in five Texas metroplexes to be $581,424,292. In agriculture, *S. invicta* have been shown to reduce soybean yield (Lofgren and Adams 1981), interfere with combine operations, interfere with root systems of plants, and feed on young growth of crops such as citrus, corn, okra, and cucumber among others (Jetter et al. 2002). Ecologically, *S. invicta* can displace native ants through resource competition and predation, (Morrison and Porter 2003; Calixto et al. 2007a, 2007b; Porter and Savignano 1990) and threaten other arthropod species. They also pose a threat to organisms such as mollusks, reptiles, birds, amphibians, and mammals (Porter and Savignano 1990; Willcox and Giuliano 2006).

Control of *S. invicta* colonies can be difficult due to this species' biology and behavior. Methods such as mound drenches or the mechanical removal of mounds provide some control, but these methods do not always affect the queen(s) and may not eliminate the colony. Even when colonies are completely eliminated, the void left by one colonies' demise constitutes an opportunity for a new colony to move in and take its place (Collins and Sheffrahn 2008; Tschinkel 2006). One effective control measure involves the use of toxic baits, which most often consist of corn grit carriers coated with soybean oil and a toxicant. These baits take advantage of foraging and food sharing by *S. invicta,* the latter known as trophallaxis. Banks (1990) states effective baits against *S.*

invicta must exhibit delayed toxicity so a large portion of the colony can receive toxicant, must be effective over a ≥10-fold range due to dilution during trophallaxis, and cannot repel ants. In addition to the aforementioned qualities, Hooper-Bui et al. (2002) observed that several urban ant species, including *S. invicta*, preferred particles of certain sizes.

Two different bait matrices were used for the following experiments: 1) Advance® Select Granular Ant Bait, formulated with soy bean oil, proteins, and carbohydrates, and 2) Advance® Granular Carpenter Ant Scatter Bait, formulated with the same ingredients along with meat meal and sugar, The purpose of these experiments was to test the following three hypotheses: 1) Mean amount of bait removed and number of ants present will not be different between particle sizes or between the two baits, 2) Starvation will not affect particle size selection or number of ants foraging, and 3) Ants with larger head capsule widths will remove larger bait particle sizes.

MATERIALS AND METHODS

Stock colonies. *Solenopsis invicta* colonies were field-collected from the USDA-ARS Pecan Breeding Orchard (N30°37'21''W96°21'34''), in Brazos County, TX. Colonies were excavated into 18.93 L grey plastic buckets lined with talcum powder to prevent escape, and transported to the laboratory, where they were separated from soil in a manner consistent with Drees et al. (2007). Colonies were placed into 40 x 27 x 9.5 cm plastic sweater boxes ([Fig. 1]: First Phillips Manufacturing, Leominster, MA) the interior wall of which were lined with Fluon® (Northern Products, Inc., Woonsocket, RI) to prevent escape (Furman and Gold 2006). Sweater boxes contained the following: 1) one 14 x 2.5 cm Petri dish, 2) two 7.5 x 2 cm plastic weigh dishes, and 3) one 4 x 0.8 cm plastic weigh dish. The Petri dish was filled with 1.5 cm of Castone® Dental Stone (Dentsply International, York, PA) and was moistened prior to placing the ants into the sweater box to serve as an artificial nest. Petri dish lids had two 3-cm holes cut into them to allow colonies access to the artificial nest. One large weigh dish contained three cotton balls saturated with water while the other contained frozen crickets (Orthoptera: Gryllidae). The smaller weigh dish contained a cotton ball saturated with a 10% honey water solution. Artificial lighting was provided at a 8:16 (L:D) h, laboratory temperatures ranged from 24°C to 29°C, and relative humidity was 60%. Colonies were fed a diet of crickets, 10% honey water, and tap water *ad libitum*.

Bait particle size profile. For comparison to any observed size preference, 200 mL aliquots of Advance® Select Granular Ant Bait and Advance® Granular Carpenter Ant Scatter Bait were sieved. Percent of each particle size relative to total weight was determined. Additionally, the number of particles of each size per mg was calculated in order to determine the mean number of particles removed of each bait size (Table 1).

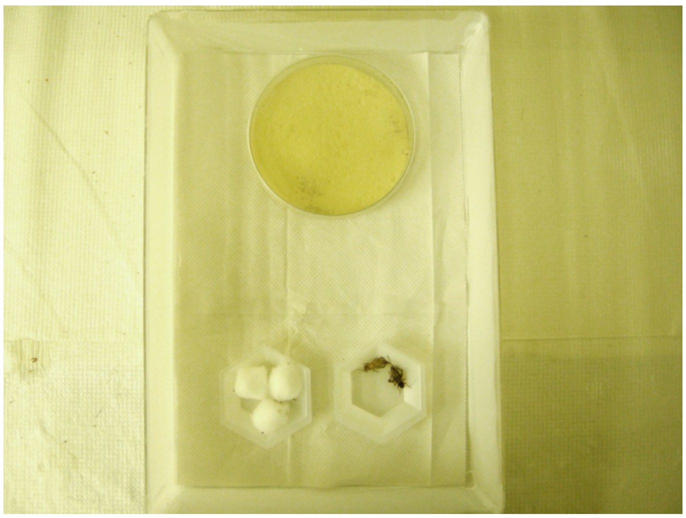

Fig. 1. A representative 40 x 27 x 9.5 cm plastic sweater box which served as artificial nest for dripped *S. invicta* colonies.

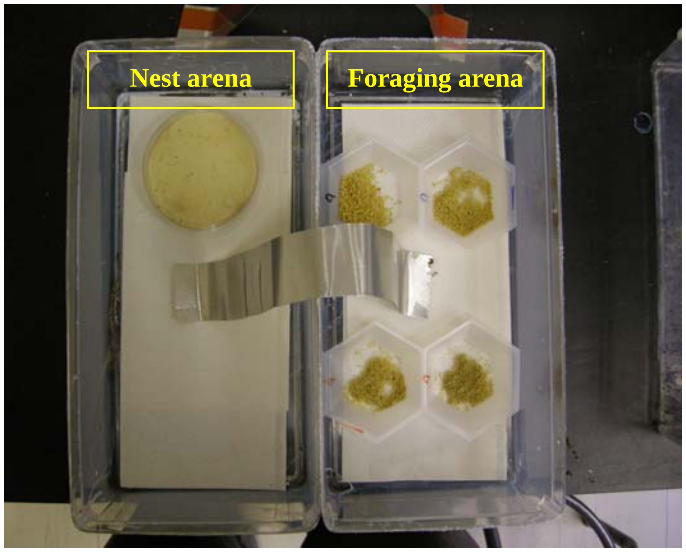

Fig. 2. Experimental colony consisted of one 21 x 16.5 x 9.5 cm plastic shoe box with a 9 x 1.5 cm Petri dish (nest arena) connected to another 21 x 16.5 x 9.5 cm plastic shoe box (foraging arena) with a 2” aluminum bridge.

Particle size choice test. Eleven experimental colonies, consisting of one functional queen, 8.4 g (~7000) of ants and 0.5 g (~975) of brood, were established in August 2009 (Fig. 2) by aspirating ants from stock colonies. An aspirator with a plastic vial was connected to a General Electric A-C motor (Model Number 5KH33DN16; General Electric Company) using 1/4” ID Nalgene® Premium non-toxic autoclavable tubing (Thermo Fisher Scientific, Rochester, NY). Ants were chilled for 180 s to reduce movement, aspirated, and placed into a 21 x 16.5 x 9.5 cm plastic shoe box (First Phillips Manufacturing, Leominster, MA) lined with Fluon®. Each plastic shoe box contained the following: 1) one 9 x 1.5 cm Petri dish with 0.75 cm of Castone® Dental Stone which served as an artificial brood chamber, and 2) a 7.5 x 2 cm plastic weigh dish filled with three water-soaked cotton balls. Desired particle sizes were obtained by crushing bait with a mortar and pestle and sifting. Particles retained by U.S. Standard sieve no. 14 (2000-1400 µm), 18 (1400-1000 µm), 25 (1000-710 µm), and bait that passed through no. 25 (<710 µm) were used for particle size choice test, and are referred to hereafter as: Size 1 (= <710 µm), Size 2 (= 710-1400 µm), Size 3 (=1000-1400 µm), and Size 4 (= 1400-2000 µm). Experimental colonies were fed a 10% honey water solution and 2 crickets per day, which were placed in an adjacent plastic shoe box lined with Fluon and connected to the nest arena by a 2” aluminum bridge. After a 2-d starvation period, 2 g of each particle size of PT-375 Advance® Select Granular Ant Bait (No active ingredient; BASF Corporation, Florham Park, New Jersey) was weighed and placed equidistant from the point where the bridge contacted the foraging arena. Controls consisted of 2 g of each particle size placed next to experimental colonies to detect weight gain or loss due absorption or loss of moisture. After *S. invicta* foragers made contact with bait, but before heavy recruitment, dish placement was altered. Bait was left in the foraging arena for 10 d, and the number of *S. invicta* foragers present on each particle size was recorded every 30 min, beginning at 8:30 h, for the first 450 min, and twice daily (8:30 – 16:00 h) for 14400 min (herein 10 d). The experiment was repeated for TC-206 Advance® Granular Carpenter Ant Scatter Bait (BASF Corporation, Florham Park, New Jersey). Two obvious limitations with the choice test design were that 1) Bait was highly concentrated and abundant, which doesn’t mimic field application rates, and 2) Bait removal and foraging were confounded by choice between four particle sizes.

***Solenopsis invicta* starvation and its effect on particle size choice.** To determine the effect of starvation on particle size preference, nine experimental colonies consisting of 6.0 g (~ 5000) of ants, 0.5 g (~975) of brood, and one functional queen were established. Experimental colonies were fed a 10% honey water solution and 2 crickets per day, which were placed in an adjacent plastic shoe box lined with Fluon and connected to the nest arena by a 2” aluminum bridge. Three colonies were supplied with 1 g of each particle size of PT-375 Advance® Select Granular Ant Bait, measured into a 7.5 x 2.0 cm plastic weigh dish and placed 3 cm from the aluminum bridge. Immediately after removing honey water and crickets (0-d group), three colonies were starved for 2 d (2-d group), and another 3 colonies were starved for 5 d (5-d group) before introducing bait. Controls consisted of three replications of 1 g of each bait particle size placed into a 21.0 x 16.5 x 9.5 cm plastic shoe box. After *S. invicta* foragers made contact with the dishes, but before heavy recruitment, the dishes were switched. Experimental colonies were allowed to forage for 450 min and were visually monitored to ensure mortality did not exceed 10%. The number of ants present on each bait size was recorded every 30 min for 450 min, or until all bait was removed for a single particle size. At the conclusion, all remaining ants were removed from the weigh dishes, bait was set out under laboratory conditions for 24 h, and the final weights were recorded.

Influence of *S. invicta* head capsule width on bait size removed. Ants were collected from 11 experimental colonies foraging on Advance® Select Granular Ant Bait (n = 299) and Advance® Granular Carpenter Ant Scatter Bait (n = 308) for 450 min. Ants that were bringing particles across the aluminum bridge were grabbed with soft forceps (BioQuip Products, Rancho Dominguez, CA) and placed into glass vials along with bait. Ant head widths were measured in mm using a ROK digital caliper (model no. DC – 122A; Rok International Industry Co., Shenzhen, China). Corresponding bait particles were measured across the long axis and were designated as Size 1, 2, 3, or 4.

Statistical analyses. Differences in bait removed and number of *S. invicta* foragers were analyzed within baits using a one-way analysis of variance (ANOVA), with bait size as the independent variable and bait size removal or number of foragers present as the dependent variable. Differences in bait size removal and number of foragers present was analyzed between baits using a Student's t-test. For starvation data, each starvation period and each bait size were analyzed separately for bait removal and number of ants present. Cumulative means were analyzed between starvation periods and between particle sizes using a one-way ANOVA. Head width analysis consisted of ants selected as they crossed the aluminum bridge from the foraging arena to the nest arena, and foragers were selected for both baits. Linear regressions were conducted for ants foraging on Advance® Select Granular Ant Bait and Advance® Granular Carpenter Ant Scatter Bait. The program SPSS 16.0 GP (SPSS Inc. 2007, Chicago, IL) was used for these analyses.

RESULTS

Bait particle size profile. The percentage of each size relative to a 200 mL aliquot of Advance® Select Granular Ant Bait (SGA) and Advance® Granular Carpenter Ant Scatter Bait (CAS) was determined (Table 1). The smallest particle size of SGA comprised a larger proportion of the total sample than the smallest size of CAS. The density of SGA was higher than that of CAS.

Table 1. Particle size profiles (200 mL aliquots) for Advance® Select Granular Ant Bait (SGA) and Advance® Granular Carpenter Ant Scatter Bait (CAS)

	SGA		CAS	
Particle size	Weight (g)	% of total	Weight (g)	% of total
Size 1: <710 µm	13.60	15.42	1.43	2.18
Size 2: 710-1000 µm	3.77	4.27	0.99	1.51
Size 3: 1000-1400 µm	11.43	12.96	9.73	14.85
Size 4: 1400-2000 µm	49.34	55.95	42.62	65.07
>2000 µm	10.04	11.39	10.73	16.38
Total bait	88.18	100	65.5	100
Density (g/mL)	0.441		0.328	

Particle size choice test. Final weights were corrected for weight change due absorption or loss of moisture by 2 mg of control baits (mean = 160.0 mg). *Solenopsis invicta* removed Size 4 particles of SGA significantly more than all other sizes (Table 1 and Fig. 3) over the 10-d foraging period. The mean amount of SGA Size 4 removed was >300 mg more than Size 3 (*P* = 0.014), which was the second most-removed. Significantly more of SGA Size 3 was removed than Size 1 (*P* = 0.001).

Table 2. Mean amounts (mg) (± SE) of four sizes of Advance® Select Granular Ant Bait removed in 10 d by 7000 *S. invicta*

Particle size (µm)	Amount of bait removed (± SE)[a]
Size 1: <710	126.7 ± 29.3a
Size 2: 710-1000	288.5 ± 53.4b
Size 3: 1000-1400	547.8 ± 65.8bc
Size 4: 1400-2000	869.8 ± 111.1c

[a]Experimental colonies were starved 2 d before testing. Means followed by different letters are significantly different at $\alpha = 0.05$ (*F* = 21.830; df = 3; *P* < 0.001; *n* = 44; Tukey's HSD test).

Ants provisioned with Advance® Granular Carpenter Ant Scatter Bait (CAS) removed sizes 2, 3, and 4 at a significantly higher rate than Size 1 (Table 2 and Fig. 4) (*F* = 8.830; df = 3, 40; *P* < 0.001; n = 44). The mean amount of CAS Size 3 removed was greater than Size 4 (*P* = 0.077) and Size 2 (*P* = 0.096), but differences were not significant at the 0.05 level.

The number of *S. invicta* foragers present at Size 4 of SGA was significantly higher (*F* = 4.718; df = 3, 40; *P* = 0.014) compared to other sizes through 10 d (Table 3).

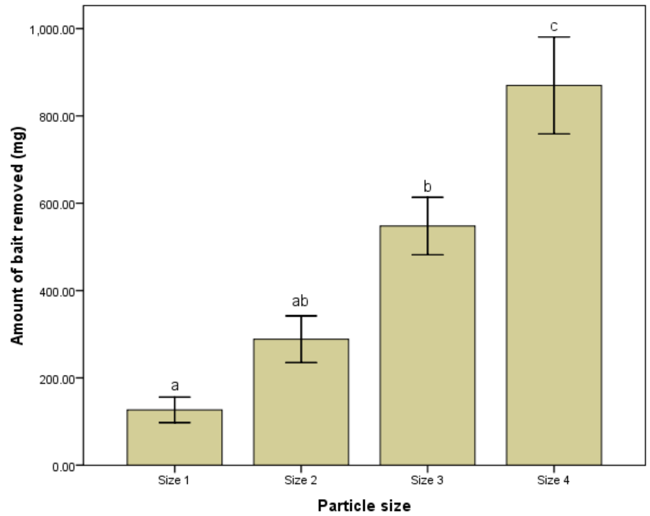

Fig. 3. Bar graph comparing mean amounts (mg) of four sizes of Advance® Select Granular Ant Bait removed in 10 d by 7000 *S. invicta*. Error bars indicate ± SE.

Table 3. Mean amounts (mg) (± SE) of four sizes of Advance® Granular Carpenter Ant Scatter Bait removed in 10 d by 7000 *S. invicta*

Particle size (μm)	Amount of bait removed (± SE)
Size 1: <710	75.3 ± 27.8a
Size 2: 710-1000	451.2 ± 103.5b
Size 3: 1000-1400	776.3 ± 128.3bc
Size 4: 1400-2000	436.8 ± 95.9c

Experimental colonies were starved 2 d before testing. Means followed by different letters are significantly different ($\alpha = 0.05$; Tukey's HSD test)

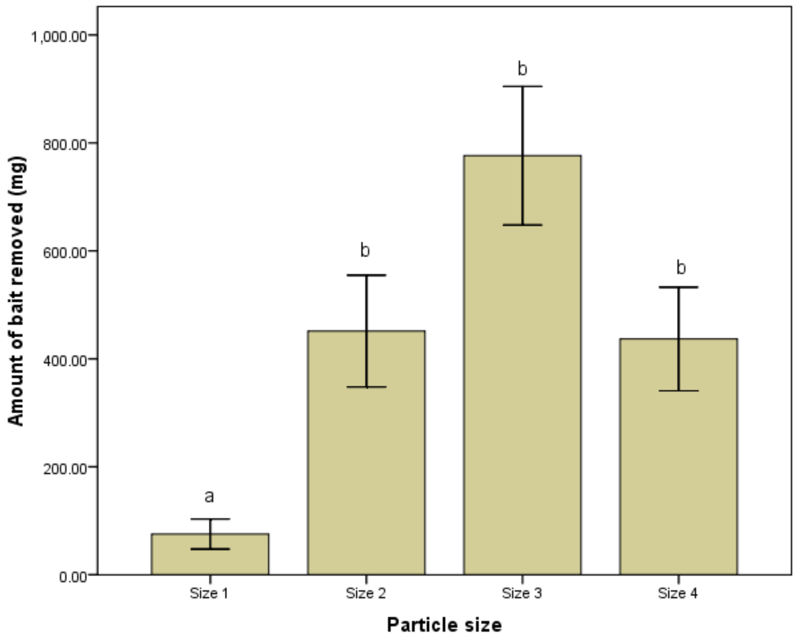

Fig. 4. Bar graph mean amounts (mg) of four sizes of Advance® Granular Carpenter Ant Scatter Bait removed in 10 d by 7000 *S. invicta.* Error bars indicate ± SE.

The mean number of ants present at different sizes of SGA for each observation is shown in Table 4 and Fig. 5. The number of ants present declined rapidly between the 450 min and 1440 min recordings, indicating satiation. The time at which the highest mean numbers of ants were present was the same for all four sizes (330 min post-introduction) and rate of recruitment (using ants present at each recording as a proxy) was surprisingly consistent between sizes for SGA. Maximum recruitment (i.e. bait that was completely covered by ants) was determined to be ~ 65 ants per dish. Differences were observed in ants foraging on different sizes of CAS (Table 5), although they differed from SGA (ants were more abundant on Size 3 than on Size 2) (P = 0.031). The mean number of ants present at different sizes of CAS for each observation is shown in Fig. 5. Like SGA, recruitment to all CAS sizes declined after 450 min, but showed a similar difference in means.

Table 4. Mean number (± SE) of *S. invicta* foragers present at four sizes of Advance® Select Granular Ant Bait over 450 min and 10 d

	No. of ants present (± SE)	
Particle size (μm)	450 min	10 d
Size 1: <710	15.9 ± 0.7a	7.4 ± 0.4a
Size 2: 710-1000	13.9 ± 0.6a	7.8 ± 0.5a
Size 3: 1000-1400	15.5 ± 0.7a	7.9 ± 0.4a
Size 4: 1400-2000	20.8 ± 0.8b	9.6 ± 0.6a

Experimental colonies were starved 2 d before testing. Means followed by different letters are significantly different ($\alpha = 0.05$; Tukey's HSD test).

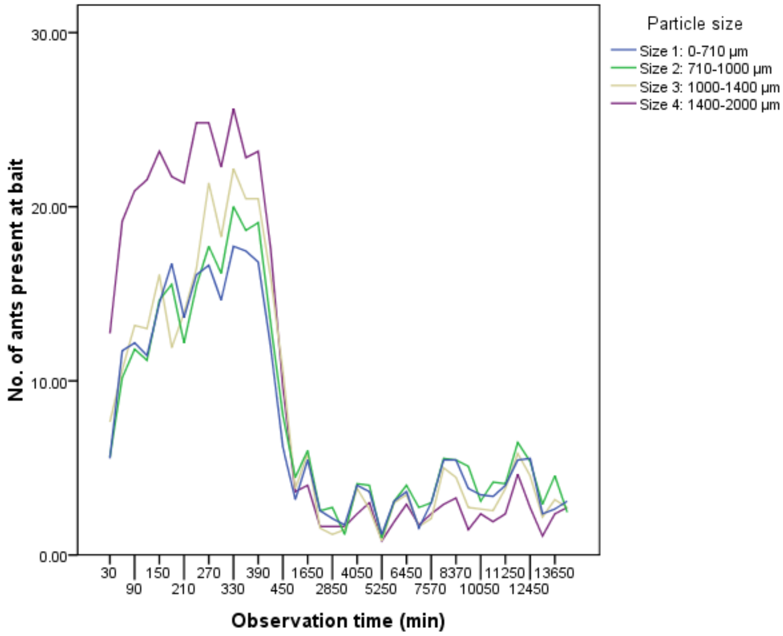

Fig. 5. Line graph indicating mean number of *S. invicta* foragers present at four sizes of Advance® Select Granular Ant Bait through 10 d.

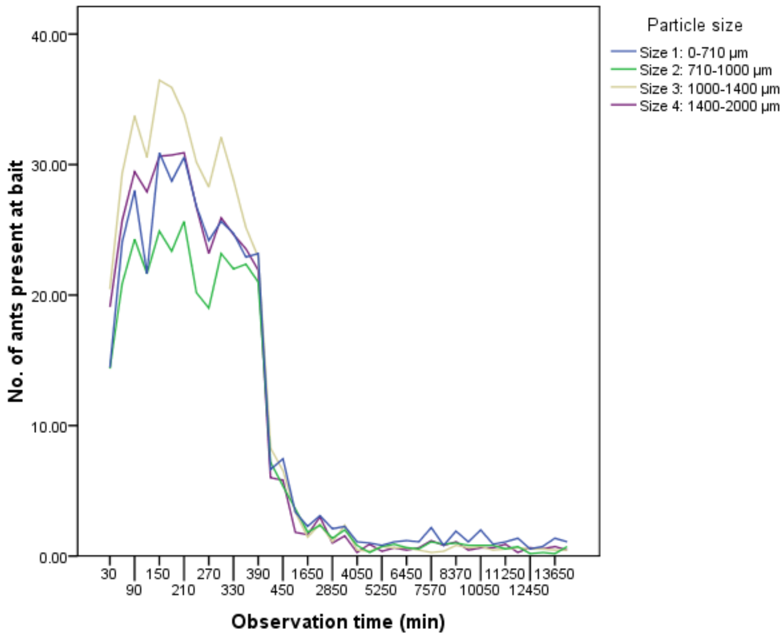

Fig. 6. Line graph indicating mean number of *S. invicta* foragers present at four sizes of Advance® Granular Carpenter Ant Scatter Bait through 10 d.

Table 5. Mean number (± SE) of *S. invicta* foragers present at four sizes of Advance® Granular Carpenter Ant Scatter Bait during 8 h and 10 d

	No. of ants present (± SE)	
Particle size (µm)	450 min	10 d
Size 1: <710	22.66 ± 1.2a	9.9 ± 0.7a
Size 2: 710-1000	19.69 ± 0.9b	8.4 ± 0.6a
Size 3: 1000-1400	26.84 ± 1.3a	11.1 ± 0.8a
Size 4: 1400-2000	23.48 ± 1.3a	9.8 ± 0.7a

Experimental colonies were starved 2 d before testing. Means in columns followed by different letters are significantly different ($\alpha = 0.05$; Tukey's HSD test).

Bait preference was determined by comparing cumulative amounts of SGA and CAS removed for all bait sizes; additionally, amounts of each bait size removed were compared between SGA and CAS. The cumulative amounts of SGA and CAS removed were not significantly different from one another (Table 6); however, Size 4 for SGA was removed at a significantly higher rate than Size 4 for CAS (t = 2.950; df = 20; P = 0.008). Bait preference based on number of *S. invicta* foragers present was determined in the same manner as bait removal. Cumulative number of ants present at CAS was significantly higher (t = 10.196; df = 1318; P < 0.001) than ants present at SGA (Table 7). CAS sizes 1, 2, and 3 had significantly greater numbers of ants present than corresponding sizes of SGA (P < 0.001 for sizes 1, 2, and 3) (note: 450 min data was used for bait preference analysis of number of *S. invicta* foragers present).

Table 6. Total amounts (mg) (± SE) of four sizes of Advance® Select Granular Ant Bait and Advance® Carpenter Ant Scatter Bait removed in 10 d by 7000 *S. invicta*

Particle size (µm)	Mean amount of bait removed (± SEM)	
	Select Granular Ant Bait	Carpenter Ant Scatter Bait
Total	458.2 ± 54.9a	434.9 ± 59.93a
Size 1: <710	126.7 ± 29.26a	75.4 ± 27.8a
Size 2: 710-1000	288.4 ± 53.43a	451.3 ± 103.5a
Size 3: 1000-1400	547.8 ± 65.85a	776.4 ± 128.3a
Size 4: 1400-2000	869.8 ± 111.1a	436.8 ± 95.9b

Means in rows followed by the different letters are significantly different (α = 0.05; Student's t-test).

Table 7. Total mean number of *S. invicta* foragers present at four sizes of Advance® Select Granular Ant Bait and Advance® Granular Carpenter Ant Scatter Bait ± SE by 7000 *S. invicta*

	No. of ants present (± SEM)	
Particle size (μm)	Select Granular Ant Bait	Carpenter Ant Scatter Bait
Total	15.9 ± 0.4a	23.2 ± 0.6a
Size 1: <710	13.5 ± 0.7a	22.7 ± 1.2b
Size 2: 710-1000	13.9 ± 0.6a	19.7 ± 0.9b
Size 3: 1000-1400	15.5 ± 0.7a	26.8 ± 1.3b
Size 4: 1400-2000	20.8 ± 0.9a	23.5 ± 1.3a

Means in rows followed by the different letters are significantly different ($\alpha = 0.05$; Student's t-test).

Table 8. Number of particles per milligram of Advance® Select Granular Ant Bait (SGA) and Advance® Granular Carpenter Ant Scatter Bait (CAS) and calculated number of particles removed following particle size choice test

	'SGA'		'CAS'	
Particle size (μm)	No. of particles per mg	No. of particles removed	No. of particles per mg	No. of particles removed
Size 1: <710	16.8	2129	22.3	1681
Size 2: 710-1000	5.6	1616	4.2	1895
Size 3: 1000-1400	2.7	1497	2.1	1630
Size 4: 1400-2000	0.9	783	0.5	218

More than twice as many particles of SGA Size 1 were removed than Size 4. In fact, as particle size increased, the number of particles removed decreased. The number of particles removed of CAS increased as particle size decreased for Sizes 4, 3, and 2, but Size 1 did not display the same trend, having fewer particles removed than Size 2.

***Solenopsis invicta* starvation and its effect on particle size choice.** Final weights were corrected for weight change due absorption or loss of moisture by 1 mg of control baits (mean = 27.5 mg). The amount removed of each particle size was compared within each starvation period (Fig. 7). The 0 d group showed a significant difference (P = 0.025) in mean amount of Size 3 removed compared to Size 1. The amount of bait removed between sizes was not significantly different in the 2 and 5 d groups, but was greater for Size 3 in both groups (P = 0.072; P = 0.05, respectively). Significantly more bait, irrespective of particle size, was removed for the 5 d group than for the 0 d group (P = 0.001), accounting for 49.3% of total mean amount of bait removed (Fig. 8). Total bait removed was significantly lower for Size 1 than the other particle sizes (Fig. 9).

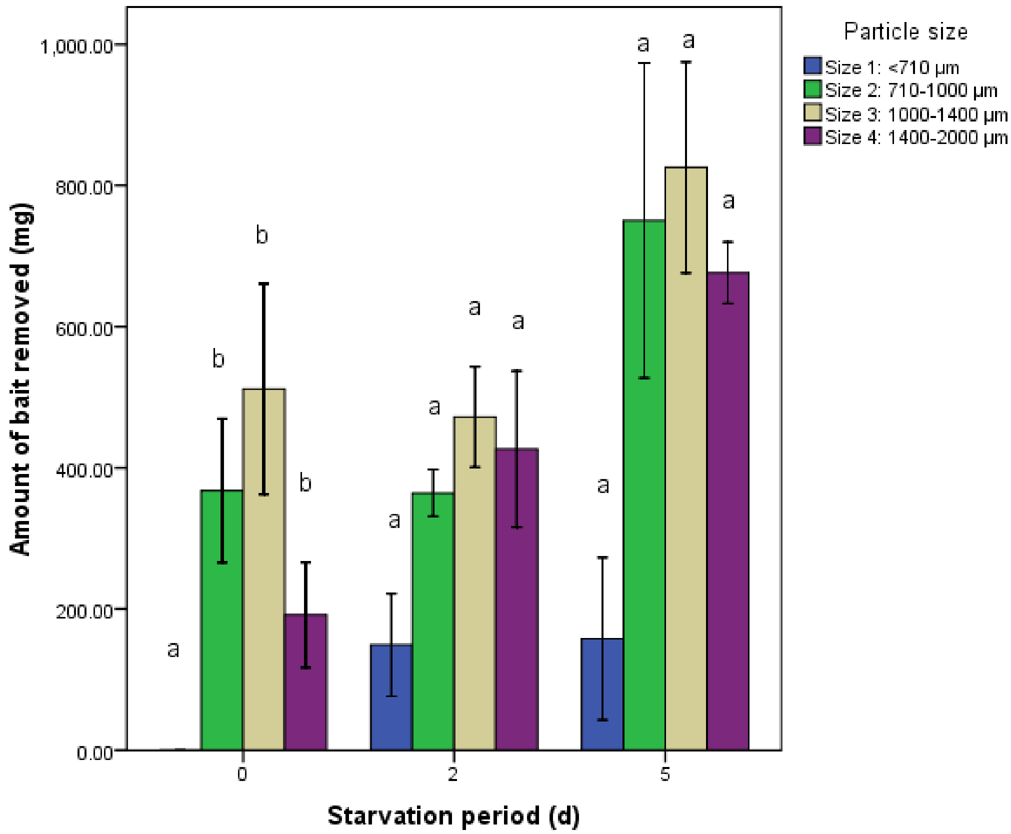

Fig. 7. Mean amounts of four sizes of Advance® Granular Carpenter Ant Scatter Bait removed (error bars indicate ± SE) by 5000 *S. invicta* for starvation periods of 0, 2, and 5 d. Different letters above columns indicate significant differences between particle sizes ($\alpha = 0.05$, Tukey's HSD test). Post-hoc comparisons were conducted for each starvation period. $F = 5.126$; df = 3; $P = 0.029$ for 0 d starvation period.

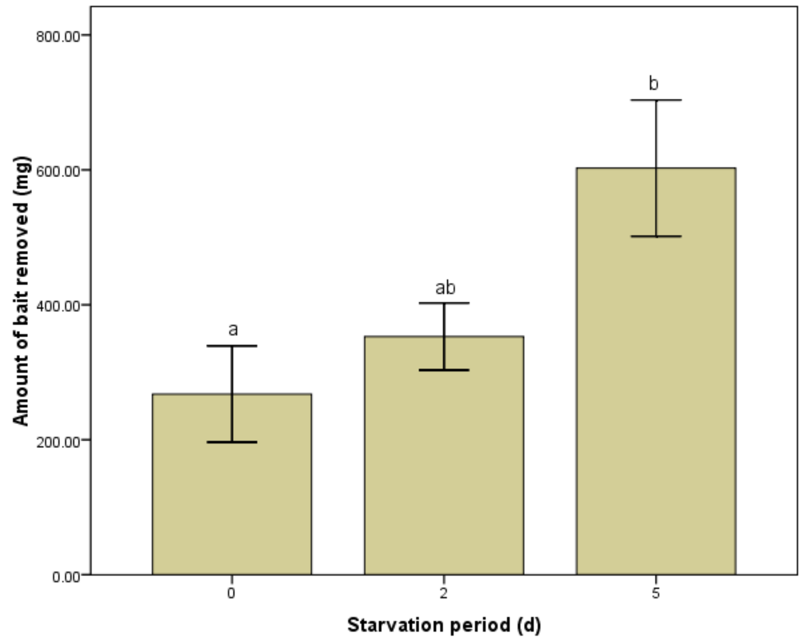

Fig. 8. Total mean amount of Advance® Granular Carpenter Ant Scatter Bait removed (error bars indicate ± SE) for starvation periods of 0, 2, and 5 d by 5000 *S. invicta* in 450 min. Different letters above columns indicate significant differences between particle sizes (F = 5.114; df = 2; P = 0.012; α = 0.05, Tukey's HSD test).

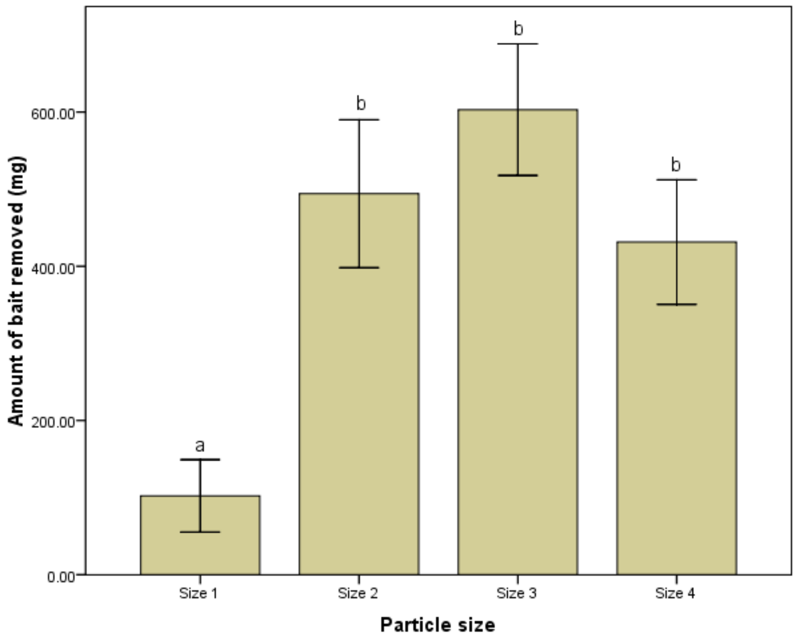

Fig. 9. Total mean amount of Advance® Granular Carpenter Ant Scatter Bait removed (error bars indicate ± SE) for different particle sizes by 5000 *S. invicta* in 450 min. Different letters above columns indicate significant differences between particle sizes (F = 7.374; df = 3; P = 0.001; α = 0.05, Tukey's HSD test).

Table 9. Number of particles of Advance® Granular Carpenter Ant Scatter Bait removed by 5000 *S. invicta* over 450 min for starvation periods of 0, 2, and 5 d

Particle size (µm)	No. of particles per mg	No. of particles removed		
		0 d	2 d	5 d
Size 1: <710	22.3	0	3323	3454
Size 2: 710-1000	4.2	1545	1529	3151
Size 3: 1000-1400	2.1	1074	991	1734
Size 4: 1400-2000	0.5	96	213	338

The total number of particles removed was similar between 0 and 2 d starvation for Sizes 2 and 3 (Table 9). Size 4 increased over 2-fold, while Size 1 increased from no particles removed at 0 d to 3323 particles removed at 2 d. The number of particles removed of each size was higher in the 5 d starvation group than the 0 and 2 d group.

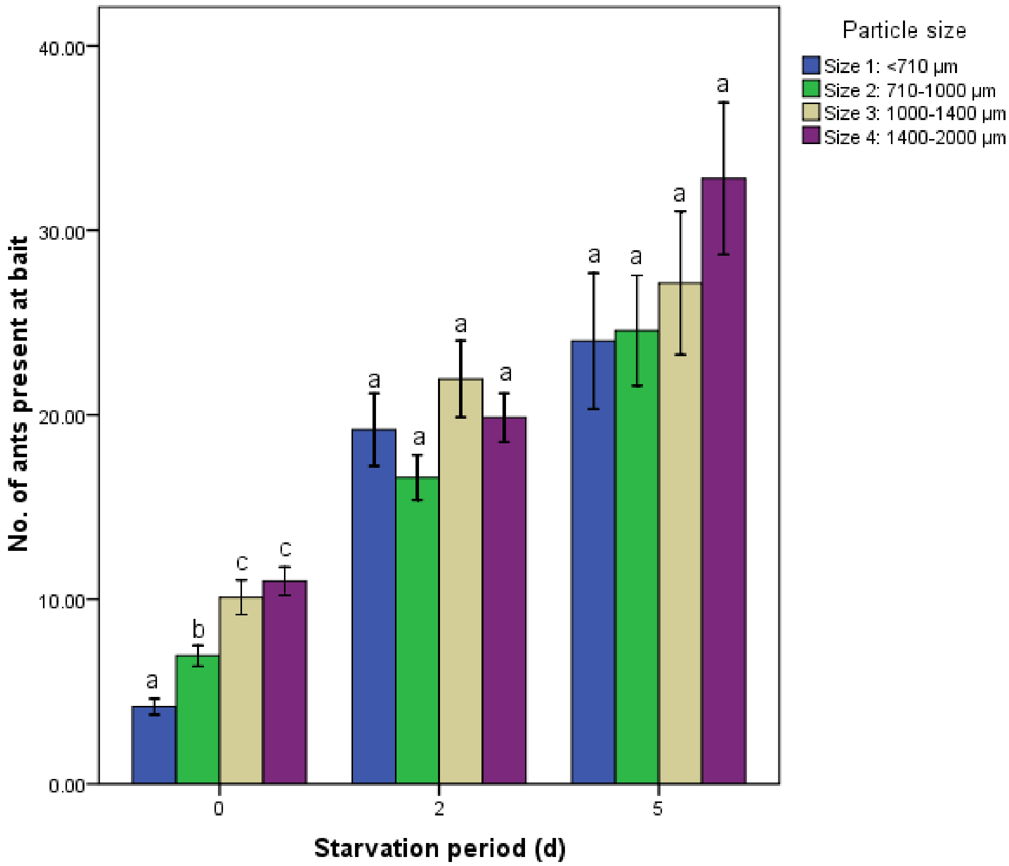

Fig. 10. Mean numbers of *S. invicta* foragers present at four sizes of Advance® Granular Carpenter Ant Scatter Bait for starvation periods of 0, 2, and 5 d. Different letters above columns indicate significant differences between particle sizes (0 d: F = 19.379; df = 3; P < 0.001; α = 0.05, Tukey's HSD test).

The mean number of *S. invicta* foragers present at bait sizes were compared within each starvation period (Fig. 10). Means were significantly different for the 0 d group, where the number of ants present was highest for the 2 largest bait sizes. Ants were present at Sizes 3 and 4 significantly more than Size 2 or 1 ($P < 0.001$). Size 2 had significantly more ants present than Size 1 ($P = 0.030$). No significant differences were observed in the 2 or 5 d groups. The total number of foragers present for starvation periods, irrespective of particle size, were highest for colonies starved for 5 d (Fig. 11). The total number of foragers were significantly more abundant on Size 4 compared to Size 1 (Fig. 12).

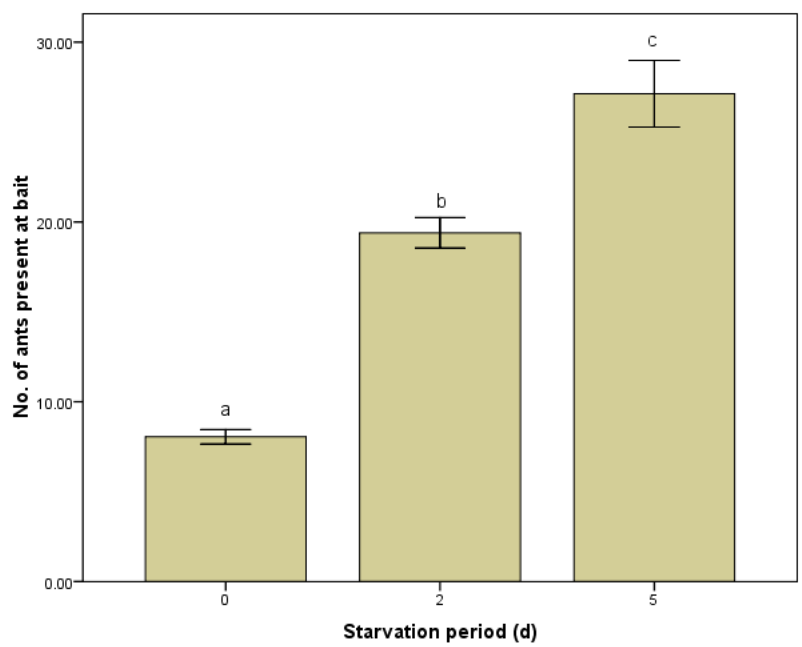

Fig. 11. Total numbers of *S. invicta* foragers present through 450 min (error bars indicate ± SE) at Advance® Granular Carpenter Ant Scatter Bait for starvation periods of 0, 2, and 5 d. Different letters above columns indicate significant differences between particle sizes ($F = 100.324$; df = 2; $P < 0.001$; $\alpha = 0.05$, Tukey's HSD test).

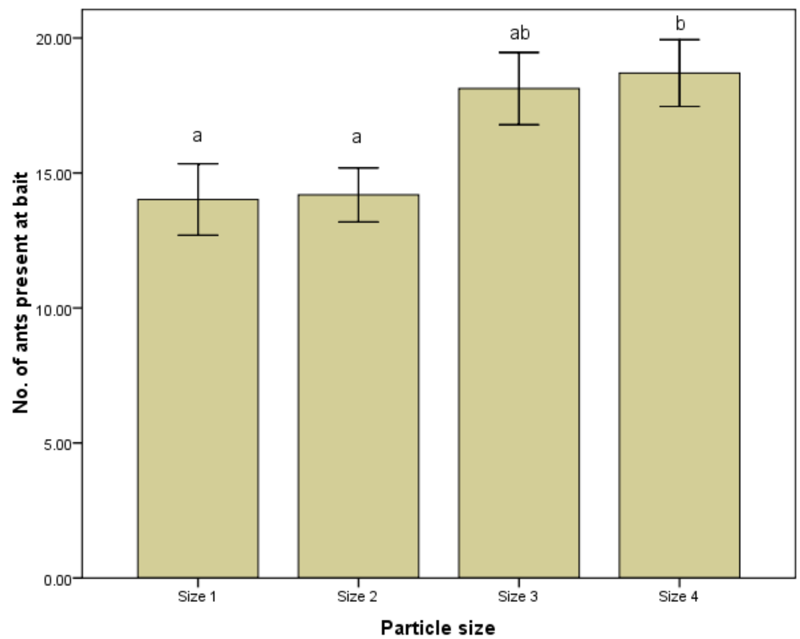

Fig. 12. Total mean number of *S. invicta* foragers present through 450 min (error bars indicate ± SE) at different particle sizes of Advance® Granular Carpenter Ant Scatter Bait. Different letters above columns indicate significant differences between particle sizes (F = 7.374; df = 3; P = 0.001; α = 0.05, Tukey's HSD test).

Influence of *S. invicta* head capsule width on bait size removed. There were wide variations in head widths of *S. invicta* foraging on SGA and CAS (0.56 – 1.25 mm; 0.57 – 1.37 mm, respectively). The mean forager head width was 0.83 ± 0.14 (std. dev.) mm for SGA and 0.82 ± 0.13 (std. dev.) mm for CAS. A linear regression model of *S. invicta* head capsule width and bait size selected was performed for both SGA and CAS.

Head capsule widths for S. invicta foragers on SGA indicated an association between head width and particle size selected ($F = 14.546$; df = 1; $P < 0.001$; slope = 0.216; R2 = 0.047; $n = 299$) (Fig. 13), and is represented by the following model: $Y = 1.609 + 0.216\ x$, where Y is particle size selected and x is worker head width. Although the low P - value for ANOVA and Student's t-test indicated *S. invicta* head capsule width had an impact on particle size selected ($t = 3.814$; $P < 0.001$), only ~ 4% of the variance in particle size selected could be accounted for by worker head capsule width when using the linear model ($R^2 = 0.043$). Head widths for *S. invicta* foraging on CAS also indicated an association between head width and particle size selected ($F = 15.835$; df = 1; $P < 0.001$; slope = 0.222; $R^2 = 0.047$; $n = 308$), and are explained by the following equation: $Y = 1.835 + 0.222\ x$, where Y is particle size selected and x is worker head capsule width in mm ($t = 3.979$; $P < 0.001$) (Fig. 14). In general, larger ants removed larger bait particles, according to the model.

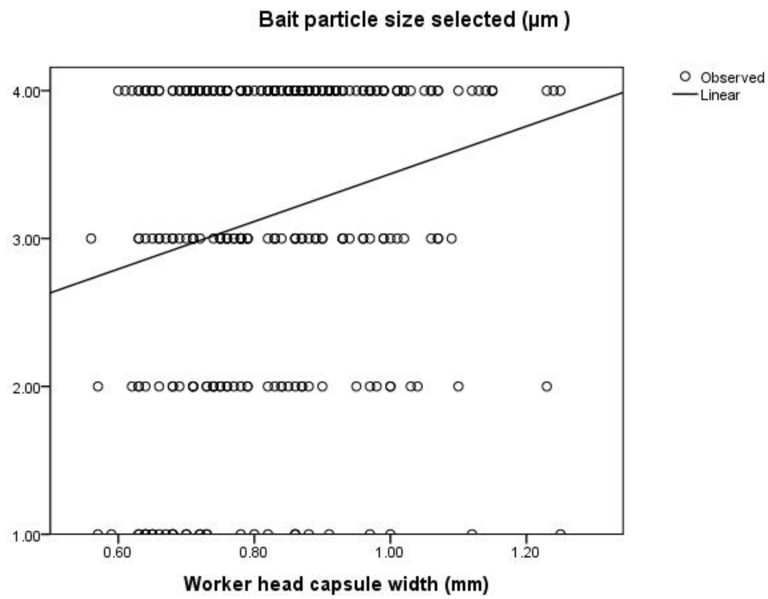

Fig. 13. Regression analysis of the influence of head capsule width on particle size removed by *S. invicta* foraging Advance® Select Granular Ant Bait. Numbers 1, 2, 3, and 4 on the *Y* – axis correspond to particle sizes 1, 2, 3, and 4, respectively.

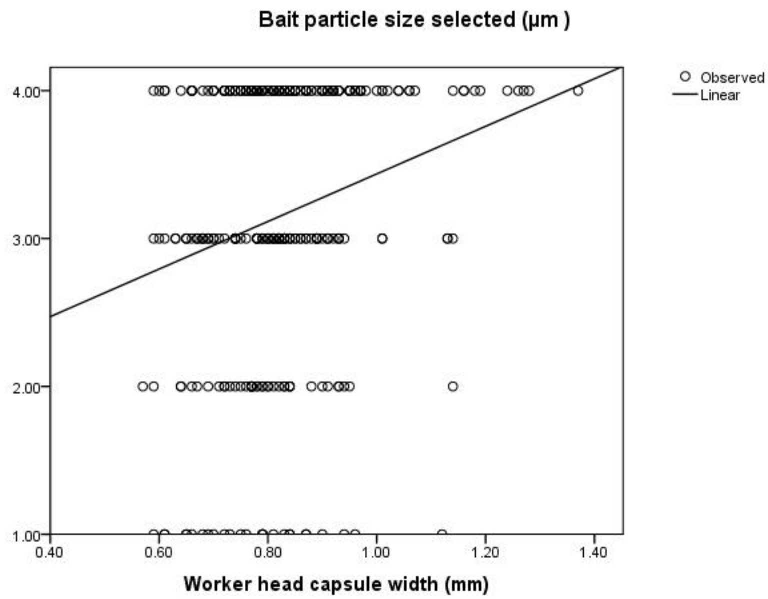

Fig. 14. Regression analysis of the influence of head capsule width on particle size removed by *S. invicta* foraging Advance® Granular Carpenter Ant Scatter Bait. Numbers 1, 2, 3, and 4 on the *Y* – axis correspond to particle sizes 1, 2, 3, and 4, respectively.

DISCUSSION

Particle size choice test. These results support previous studies in which ants preferred larger particle sizes, based on amount and number of particles removed (Hooper and Rust 1997, Hooper-Bui et al. 2002). Surprisingly, this preference was not the same between the two baits used in the experiment, suggesting particle size preference may be bait-specific. *Solenopsis invicta* foragers showed a distinct preference for the largest particle size (Size 4) of Advance® Select Granular Ant Bait (SGA) based on amount of bait removed and number of ants present. While foragers removed more of, and were present in higher numbers at Size 3 of Advance® Granular Carpenter Ant Scatter Bait (CAS), means could not be separated between sizes 1, 2, and 4. Even so, in 6 of the 11 colonies foraging on CAS, Size 3 was the most-removed, and accounted for 44.6% of the total amount of CAS removed. Size 4 was the most-removed particle size of SGA in 8 of the 11 colonies, and accounted for 47.5% of total SGA removed. Sizes 3 and 4 represented 77.4% and 69.7% of total SGA and CAS removed, respectively. The finding that mean amount of bait removed and mean ants present both indicated a preference for larger particles contrasts with Hooper-Bui et al. (2002) where *S. invicta* removed more bait of larger particle sizes (>2000 µm) but visited smaller particle sizes (840-590 µm) more often.

This study differed from previous ones in that the number of ants present was used in place of particles removed. The number of particles removed generally increased as particle size decreased, with the exception of CAS Size 1 (Table 8), which is similar to the findings of Hooper-Bui et al. (2002). The number of particles removed of each bait may provide valuable information from a control perspective. If toxicant is coated on the outside of the bait, then the surface area to volume ratio determines how much toxicant is being administered into the colony, but if the toxicant is absorbed into the corn grit, the amount of toxicant would increase proportionally with an increase in volume.

The most important factor in broadcast bait efficacy is the distribution of active ingredient among ants, so any modification of bait properties (i.e. size, attractant, matrix, etc.) should be designed to maximize the amount of active ingredient taken by ant colonies. Whether this is accomplished by removing a larger number of smaller particles or by removing fewer large particles remains to be tested. The goal of this study was to determine if ants displayed a preference for different sizes of bait, so number of foragers present at baits may give a better indication of preference because it is a measurement of recruitment to the bait. Hangartner (1969) demonstrated that ants secrete trail pheromone in relation to food profitability, with vigor and quantity more defined for higher-quality foods. Trail pheromone dissipates within a few minutes, so in order to maintain a viable trail, foraging ants must reinforce it by laying their own trial on top of the existing one. This can only be accomplished if a foraging ant inspects the food source, so higher-quality foods illicit a stronger trail because more recruits choose to add to the existing trail (Tschinkel 2006). The more recruits that encounter a food source and add to the trail pheromone, the faster the trail builds up and the more workers are drawn to the food source. Thus, the number of ants present at the food source should serve as an appropriate proxy for particle size preference, but this scenario is not without its own limitations. Bait was extremely abundant, with 8000 mg of bait in a 379.5 cm^2

foraging arena. If the label rate of 1.5 lbs per ha was used, the same foraging area would have only 0.000015 mg of bait. Some foraging ants simply walked across the bait surface, neither feeding nor removing bait, which would result in no control in a field situation. Lack of foraging area may have contributed to these results, or simply that some ants did not determine the bait to be a quality food source.

Ants were overall more abundant on CAS than on SGA. Interestingly, this did not translate to an increase in the amount of bait removed. According to Lopez et al. (2000), Advance® Select Granular Ant Bait is formulated with soybean oil, although according to Whitmire - Micro-gen®, a unique blend of proteins and carbohydrates are used as an attractant. Advance® Granular Carpenter Ant Bait (the most similar product to bait tested in this study) uses the same ingredients except for the addition of meat meal and sugar, which may increase the quality of the bait to *S. invicta* foragers. Lopez et al. (2000) determined the preference and efficacy of several toxic baits, including Advance® Select Granular Ant Bait and Advance® Granular Carpenter Ant Bait, the latter of which was most preferred and provided the best control of the mound-building ant, *Lasius neoniger* Emery. Bait preference was determined by comparing the amount of bait removed and number of ants present between the two baits during independent trials. This removed the confounding factor of bait choice from the experiment, making preference measured absolute for each bait type. Additional experiments should be conducted that assess particle size preference in this manner.

Optimum foraging theory suggests foraging ants should maximize net energy intake per unit feeding time, which would correspond to removing the largest bait particles possible (Bailey and Polis 1987, from Hooper-Bui et al. 2002). Control can also vary between bait sizes, depending on the ability of the bait to reach target ant populations. Often, broadcast baits are distributed via plane or helicopter over large land areas. Smaller particles, with a smaller surface area to volume ratio, are more affected by wind drift than larger particles, which have a comparatively lower ratio. If baits are deposited off-site or remain atop grasses and other plants, control may be significantly reduced compared to larger bait sizes that have enough mass to reach the ground.

***Solenopsis invicta* starvation and its effect on particle size choice.** This study found that satiated *S. invicta* were more selective of particle sizes, and this selectivity was negatively correlated with starvation length, especially in terms of the number of ants present at bait. This is supported by Bailey and Polis (1987), who found that satiated California harvester ants, *Pogonomyrmex californicus*, decreased their foraging intensity and harvested fewer types of prey compared to similar experimental colonies of starved ants. In our study, ants demonstrated degrees of bait size selectivity which correlated to a continuum of starvation severity. That is, the longer they were starved, the less selective they were regarding the size of baits selected, based on number of ants present (Table 9), a trend that is supported by Hangartner (1969). In addition to food quality, Hangartner (1969) showed that hunger dictates the vigor and quantity of trail marking. Because ants starved for longer periods of time should lay stronger trails to any food source encountered, it may be assumed that the number of ants present would not be different for colonies that had been starved longer, since any food encountered would be heavily recruited to.

CAS was the only bait used for starvation experiments due to insufficient amounts of SGA available. Despite this setback, results can be compared to results from particle size choice tests to determine if observed particle size preference is the same with the additional variable starvation length. Interestingly, despite lack of statistically significant differences between the amounts of bait removed at different particle sizes, graded preference based on mean amounts of bait removed was similar (Size 2 > Size 3 > Size 1 > Size 4) to graded preference for the particle size choice test of CAS. While this provides no evidence to support the alternative hypothesis that starvation effects particle size selected, it might be inferred that particle size preference is more related to bait composition and palatability than to other variables, such as starvation or prior feeding. More replications should be included, along with trials including SGA, to further investigate the effect of starvation on particle size preference. Additional experiments, including ones that account for prior feeding and other available foods, should be conducted as well.

Influence of *S. invicta* head capsule width on bait size removed. Forager head capsule width was a good predictor of the size of particle removed for both SGA and CAS, but the predictive value for the linear model was low ($R^2 = 0.043$; $R^2 = 0.047$, respectively). Other models were used to try and obtain a larger R^2 – value, but none could explain more than ~4 % of the variance observed (data not shown). One possible reason for such a weak linear correlation might be due to particle size measurement. Determining a standard area of the bait to measure proved extremely difficult because of particle variability in size and shape, so baits were placed into appropriate size categories based on long-axis measurements, which may have affected the predictive capacity of the regression. Median values for particle sizes removed were consistent with preferred sizes determined by bait removal and foraging. Observations made while selecting ants from the aluminum bridge showed that even small workers could remove larger particles, without the help of additional foragers. This is consistent with Hooper and Rust (1997) and would explain the large amount of removal of particle sizes 1 (SGA) and 2 (CAS).

Mean head capsule widths for ants foraging on both baits correspond with head widths observed for monogyne workers, which were 0.88 ± 0.21 and 0.87 ± 0.37 mm (for colonies from Georgia and Texas, respectively) (Greenberg et al. 1985). However, the presence of multiple de-alated females (possible polygyne queens) contradicts this. Greenberg et al. (1992) goes on to classify colonies as "intermediate" (unknown if monogyne or polygyne) if worker head widths are between 0.737 and 0.841 mm, and a study in Louisiana (Colby et al. 2007) found considerable overlap between the two forms. Classification as either monogyne or polygyne is further confounded because only a small proportion of the experimental colony worker population (foragers returning with bait) was analyzed.

Worker size affects the division of labor in a fire ant colony (Cassill 2003, Tschinkel 2006), as well as the length of each task performed by *S. invicta* workers. Major workers groom and feed larvae less frequently than medium or small workers, but account for a large portion of the food retrieval force. In addition to size, worker age, to some degree, dictates the type of work performed by an individual ant, with older workers comprising a majority of the foraging body. However, age and body size do not fully explain the division of labor. Tofts (1993) proposed the "foraging for work" hypothesis, stating that workers emerge from the pupa in the brood pile, and begin searching for work, with brood care being the first work they encounter. Gradually, workers move away from the brood pile and find other tasks, eventually ending up outside the nest as foragers.

SUMMARY AND CONCLUSIONS

Based on results from this study, bait removal and foraging activity are influenced by bait particle size. Preferred bait sizes should be incorporated into baits labeled for fire ant control, but this study suggests different baits should be analyzed in a similar fashion, as preference of a specific size for a type of bait may not confer preference to the same size of other baits. While no difference was observed in total removal of SGA or CAS, *S. invicta* foragers were more abundant on CAS, which may have important control implications.

As starvation time increased, total bait removal and number of ants present on different sizes of CAS increased. Satiated ants showed more selectivity to a preferred particle size, while hungry ants were not nearly as selective. Both bait removal and foraging were positively correlated with starvation length, but showed similar ratios of particle sizes preferred. The size preference observed corresponded to data from particle size choice test, further bolstering the argument that size preference may be bait-specific.

Forager head capsule width was positively correlated with the size of bait removed for both SGA and CAS, but not in a linear fashion. The fact that even small foragers were able to remove large bait sizes suggests larger sizes are indeed preferred. Median values for particle size removed were consistent with particle size preferred based on amount of bait removed and number of foragers present for SGA and CAS. Social form could not be accurately identified by mean head widths from sample populations based on Greenberg et al. (1992), and fell within an intermediate size range.

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