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

by

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ABSTRACT

One of the most effective methods for achieving control of red imported fire ants, *Solenopsis invicta* Buren is through the application of broadcast baits. Multiple factors contribute to bait efficacy, one of which is particle size. Bait mass removed, the number of particles removed, and the number of ants present at dishes containing bait of a specific particle size were recorded and used to determine bait and particle size preference of foraging *S. invicta*. Additionally, head capsule widths of foraging ants were measured and compared to particle size removed. The mean mass of bait removed by foraging ants was significantly greater ($P < 0.05$) for 1400-2000 µm particles of Select Granular Ant Bait (SGA), but more total particles of bait <710 µm were removed. The mean mass of Advance Carpenter Ant Scatter Bait (CAS) removed was significantly less for particles <710 µm, while foragers removed more 710-1000 µm particles. Significantly more ants were present at SGA particles 1400-2000 µm, while ant counts on CAS were significantly lower for 710-1000 µm particles. Mean head capsule width of foraging ants returning with SGA 1400-2000 µm particles were significantly wider than those returning with <710 µm particles. For CAS, mean head capsule width of ants returning with 1400-2000 µm particles were significantly wider than those returning with <710 µm or 710-1000 µm particles. Implications for control of *S. invicta* populations are discussed.

Key Words: particle size preference, bait preference, *Solenopsis invicta*.

INTRODUCTION

Solenopsis invicta is an invasive ant species native to South America, most likely originating in the Mesopotamia flood plain near Formosa, Argentina

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(Tschinkel 2006; Caldera *et al.* 2008). Since it arrived in Mobile, Alabama roughly 75 years ago, *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 22,662,396 ha in Texas, with 160 counties under quarantine (Lard *et al.* 2002). 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 approximately $600,000,000. These ants are major agricultural and urban pests throughout much of the southeastern United States. In agriculture, *S. invicta* have been shown to reduce soybean yield (Lofgren & 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). *S. invicta* can displace native ants through resource competition and predation, (Morrison & Porter 2003; Calixto *et al.* 2007a, 2007b; Porter & Savignano 1990), and threaten other arthropod species. They also pose a threat to organisms such as mollusks, reptiles, birds, amphibians, and mammals (Porter & Savignano 1990; Willcox & Giuliano 2006).

Control of *S. invicta* colonies can be difficult due to the biology and behavior of this species. Methods such as mound drenches or the mechanical removal of mounds provide some control, but these do not always affect the queen(s), and may not eliminate the colony. In addition, these methods are extremely labor intensive. Even when colonies are eliminated, the void left behind constitutes an opportunity for a new colony to move in and take its place (Collins & 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 trophallaxis by *S. invicta*. Stringer *et al.* (1964) indicated that effective *S. invicta* baits must: 1) exhibit delayed toxicity so a large portion of the colony can receive toxicant; 2) must be effective over a ≥10-fold range due to dilution during trophallaxis; and, 3) cannot repel ants. In addition to the aforementioned qualities, Hooper-Bui *et al.* (2002) observed that several urban ant species, including *S. invicta*, preferred bait particles of specific 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. Both commercially-sold baits contain the active ingredient Abamectin, derived from the soil bacterium *Streptomyces avermitilis*. Abamectin activates both γ-aminobutyric acid (GABA) chloride channels and ibotenate-activated glutamate receptors, resulting in an inhibitory effect with symptoms of ataxia and eventual paralysis (Bloomquist 1993). For these experiments, baits were formulated without active ingredient. The purpose of this research was to test the following hypotheses: 1) Mean amount of bait removed and number of ants present will not be different between particle sizes or between the two baits; and, 2) ants with larger head capsule widths will remove larger bait particle sizes.

MATERIAL 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 18.9 L plastic buckets lined with talcum powder to prevent escape, and transported to the Center for Urban and Structural Entomology (Texas A&M University) 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 (First Phillips Manufacturing, Leominster, MA) the interior walls of which were lined with Fluon® (Northern Products, Inc., Woonsocket, RI) to prevent escape (Furman & 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 weigh dish contained three cotton balls saturated with water, while another 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 8:16 h (L:D) , laboratory temperatures ranged from 24°-29°C, and relative humidity was 60 (±2%). Colonies were fed a diet of crickets, 10% honey water, and water *ad libitum*.

Bait particle size profiles.

For comparison to any observed size preference, 200 mL 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 gram was calculated in order to determine the mean number of particles removed of each bait size.

Particle size choice test.

Eleven experimental colonies, consisting of one functional queen, 8.40 g (~7000) of workers and 0.50 g (~975) of brood, were established in August 2009 by aspirating ants from laboratory 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® (Northern Products, Inc., Woonsocket, RI). 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.

Specific 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 the 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 two crickets per day, which were placed in an adjacent plastic shoe box lined with Fluon®, and connected to the nest arena by a 5.1 cm 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. Untreated controls consisted of 2 g of each particle size placed next to experimental colonies to detect weight gain or loss due to 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 then twice daily (8:30 – 16:00 h) for 10 d. The experiment was repeated for TC-206 Advance® Granular Carpenter Ant Scatter Bait (BASF Corporation, Florham Park, New Jersey).

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 collected with soft forceps (Bio-Quip Products, Rancho Dominguez, CA), and then 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 the amount of bait removed and the 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 the bait size removal and the number of foragers present were 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 from both baits. Head width of each ant was measured and the corresponding particle size they returned with were recorded, and a one-way ANOVA was conducted to determine if mean head capsule widths were different for ants returning with different particle sizes. The level of significance of all statistical analyses was $P < 0.05$. The program used for these analyses was SPSS v. 16.0 GP (SPSS 2007, Chicago, IL).

RESULTS

Bait particle size profile

The mean percentage of each particle size relative to a 200 mL aliquot of SGA and CAS was determined. The smallest particle size of SGA comprised a larger proportion of the total sample than the smallest size of CAS. The density of SGA (0.441 g/mL) was higher than that of CAS (0.328 g/mL).

Particle size choice test

Final weights were corrected for weight change due to absorption or loss of moisture, as determined by weight change of control baits. *Solenopsis invicta* removed significantly more Size 4 particles of SGA than all other sizes over the 10 d foraging period (Table 1). The mean amount of Size 4 removed was > 0.30 g more than Size 3 ($P = 0.014$), which was the second most-removed. A significantly higher amount of Size 3 was removed than Size 1 ($P = 0.001$).

There were significant differences in mean amounts of different particle sizes of SGA removed ($F = 20.679$; df = 3; $P < 0.001$). Tukey's HSD showed mass removed by foraging ants was greatest for Size 4, followed in order by Sizes 3, 2, and 1 (Table 1). Significant differences in mean mass removed of different sizes of CAS were detected as well ($F = 8.830$; df = 3; $P < 0.001$). Mean amounts removed of Sizes 2, 3, and 4 were significantly different from mean amount of Size 1 removed (Table 1). Size 3 was the most-removed, with >0.3 g more removed than Size 2, but the means were not significantly different ($P = 0.077$). While mean mass removed indicated a preference for particles > 1000 μm (Sizes 3 and 4), conversion of mass removed to number of particles removed showed a preference for Sizes 1 and 2 for SGA and CAS, respectively (Table 2). 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. This trend was not recorded in Size 1, which had fewer particles removed than Size 2 (Table 2).

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 ($t = 0.294$; df = 86; $P = 0.770$; Table 1). However, SGA Size 4 was removed

Table 1. Total amounts (g) (± 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)[1]	
	Select Granular Ant Bait	Carpenter Ant Scatter Bait
Size 1: <710	0.127 ± 0.029a,a	0.075 ± 0.028a,a
Size 2: 710-1000	0.289 ± 0.053ab,a	0.451 ± 0.103b,a
Size 3: 1000-1400	0.548 ± 0.066b,a	0.776 ± 0.128b,a
Size 4: 1400-2000	0.870 ± 0.111c,a	0.437 ± 0.096b,b
Total[2]	0.458 ± 0.055a	0.435 ± 0.060a

[1] The first letter following values corresponds to comparisons within the column. Letters following a comma signify Student's t-test comparisons of the same particle size between SGA and CAS (row comparison).
[2] Mean amount of bait removed for all particle sizes.

Table 2. Mean number of particles per gram (± SE) of SGA and CAS and calculated number of particles removed following particle size choice test.

	SGA		CAS	
Particle size (μm)	No. of particles per g	No. of particles removed	No. of particles per g	No. of particles removed
Size 1: <710	5394 ± 288	685	10110 ± 227	758
Size 2: 710-1000	2296 ± 154	663	4048 ± 175	1825
Size 3: 1000-1400	1134 ± 61	621	1578 ± 73	1224
Size 4: 1400-2000	525 ± 19	456	611 ± 70	267

Number of particles removed was calculated by multiplying the mean number of particles for each size by the corresponding amounts removed shown in Table 1.

at a significantly higher rate than CAS Size 4 ($t = 2.950$; df = 20; $P = 0.008$). Overall, total amount of SGA and CAS removed did not demonstrate ant preference for either bait (Table 1).

In ants foraging on SGA, significantly more were counted on Size 4 than all other sizes ($F = 20.187$; df = 3; $P < 0.001$). For those foraging on CAS, significantly fewer ants were counted at Size 2 particles than all other sizes. Size 3 had the highest mean number of foraging ants, followed by Size 4, then Size 1 (Table 3). 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. CAS sizes 1, 2, and 3 had significantly greater numbers of ants present than corresponding sizes of SGA ($P < 0.001$

Table 3. Mean number (± SE) of *S. invicta* foragers present at four sizes of SGA and CAS over 450 min.

	No. of ants present (± SE)	
Particle size (μm)	SGA[1]	CAS
Size 1: <710	15.9 ± 0.7a,a	22.6 ± 1.2a,b
Size 2: 710-1000	13.9 ± 0.6a,a	19.7 ± 0.9b,b
Size 3: 1000-1400	15.5 ± 0.7a,a	26.8 ± 1.3a,b
Size 4: 1400-2000	20.8 ± 0.8b,a	23.5 ± 1.3a,a
Total[2]	15.9 ± 0.4a,a	23.2 ± 0.6a,a

[1] The first letter following values corresponds to comparisons within the column using Tukey's HSD. Letters following a comma signify Student's t-test comparisons of the same particle size between SGA and CAS (row comparison).

[2] Mean numbers of foraging ants present at all bait sizes.

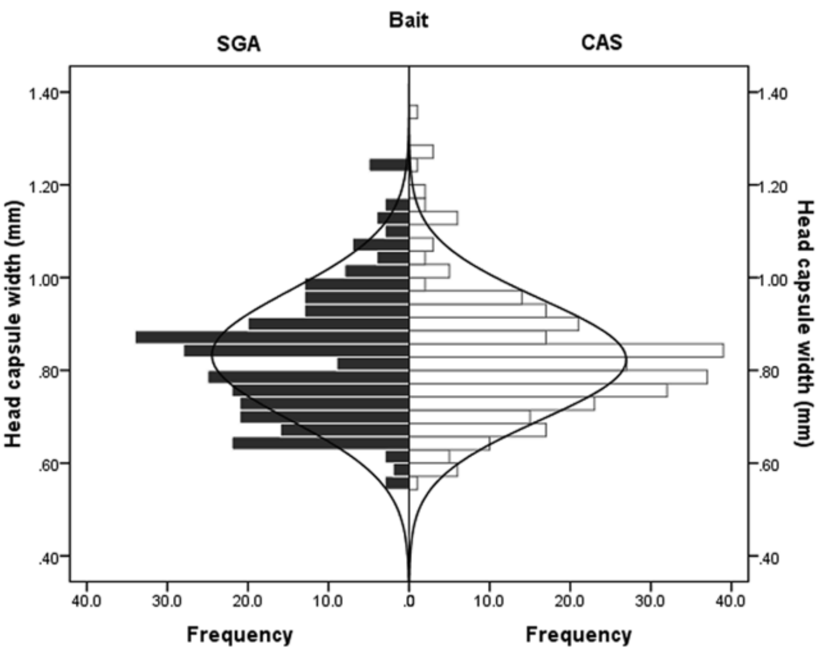

Fig. 1. Histogram of all *S. invicta* forager head widths sampled. Curves indicate normal distribution.

for sizes 1, 2, and 3) (note: 450 min data was used for bait preference analysis of number of *S. invicta* foragers present).

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; Fig. 1). The mean ant forager head width was 0.83 ± 0.14 mm for SGA and 0.82 ± 0.13 mm for CAS. For SGA, mean head capsule width for ants returning with Size 4 particles was significantly greater than those returning with Size 1 particles ($F = 4.685$; df = 3; $P = 0.003$; Fig. 2). Mean head capsule width for ants foraging on CAS was also widest in those returning with Size 4 particles than those returning with Size 1 or Size 2 particles ($F = 5.554$; df = 3; $P = 0.001$).

DISCUSSION

Particle size choice test

Optimum foraging theory suggests animals, ants in this case, should maximize net energy intake per unit feeding time, which would correspond to removing the largest bait particles possible (Bailey and Polis 1987, Hooper-Bui *et al.* 2002). The degree of pest ant control success can vary between bait sizes, depending on the ability of the applicator to introduce baits to target ant populations. Often, broadcast baits are distributed via plane or helicopter over large areas of land. Smaller particles, with a larger surface area to volume ratio and lower mass, are more affected by wind drift than larger particles, which have a comparatively lower surface to volume 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.

Based on the amount and number of particles removed, these results support previous studies in which ants preferred larger particle sizes (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 SGA based on the amount of bait removed and number of ants present. While foragers removed more of, and were present in higher numbers at Size 3 of CAS, the 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 the mean amount of bait removed and mean number of ants present both indicated a preference for larger particles, contrasts with the findings of 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.

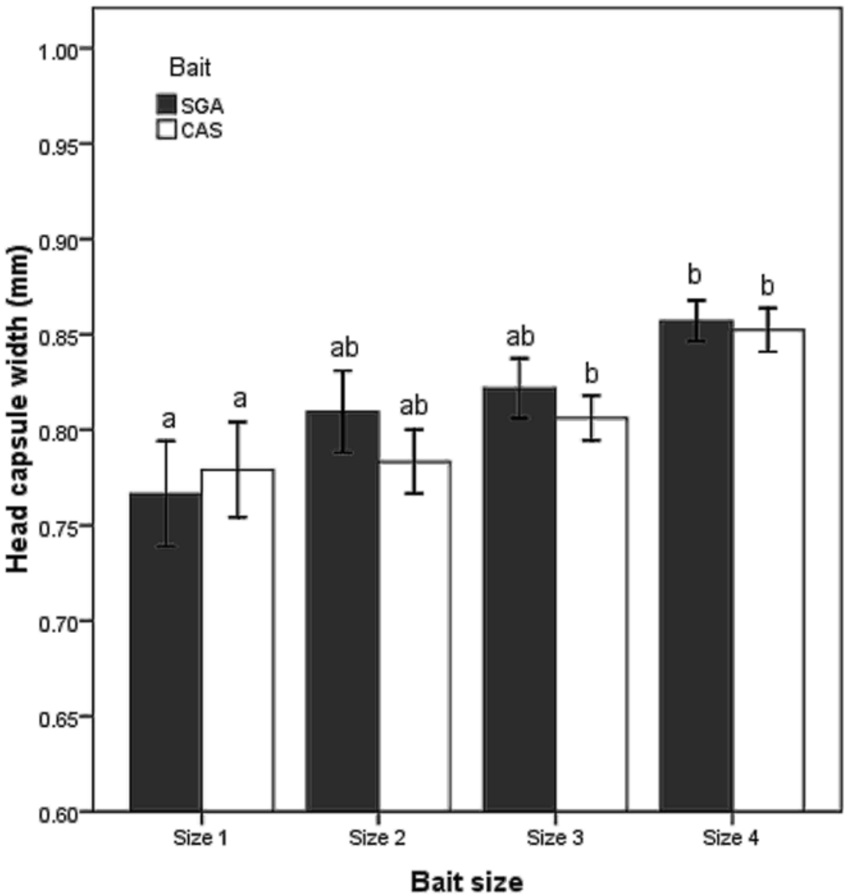

Fig. 2. Mean head capsule width (mm) of foraging *S. invicta* returning with different particle sizes. Post-hoc comparisons are between different particles sizes of the same bait. Bars of the same bait followed by different letters are significantly different (Tukey's HSD, $\alpha = 0.05$).

This study differed from previous work in that the number of ants present was analyzed in addition to particles removed. The number of particles removed generally increased as particle size decreased, with the exception of Size 1 CAS (Table 2), which is similar to the findings of Hooper-Bui *et al.* (2002). The number of particles removed may provide valuable information from a control perspective. If toxicant is coated on the outside of the bait, then the surface area determines how much toxicant is available to 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, (including size, attractant, and matrix) 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 the number of foragers present at baits through time may give a better indication of preference because such observations include, by default, 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 constant trail, foraging ants must reinforce it by laying their own trail 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 8.00 g of bait in a 379.5 cm^2 foraging arena. If the actual label rates of these products were used (0.68 kg per ha), the foraging area associated with these arena would receive only 0.01 g of bait. Some foraging ants simply walked across the bait surface, neither feeding on nor removing bait, which would result in no control in a field situation. Lack of foraging area may have contributed to these results. Additionally, some ants may have simply not determined the bait to be a quality food source. Overall, ants were 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 CAS in this study) uses the same ingredients except for the addition of meat meal and sugar, which may increase palatability of the bait to *S. invicta* foragers.

Influence of *S. invicta* head capsule width on bait size removed

Worker size affects the division of labor in a fire ant colony (Cassill 2003, Tschinkel 2006), as well as the duration 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 foraging members of the colony. In addition to size, worker age dictates the type of work performed by an individual ant, with older workers comprising a majority of the foraging cohort. 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.

For both SGA and CAS, mean head width was greater in foragers returning with larger particle sizes. This provides experimental evidence of larger ants removing larger particles, but it doesn't tell the entire story. Even ants with 0.60 mm head capsules were able to remove Size 4 particles, and foragers with 1.25 mm head capsules were observed removing Size 1 particles. A scatter plot showed foragers with head capsules up to 1.00 mm removed at least some of all particle sizes. Additionally, a trend was observed in which ants with head capsules >1.00mm removed large particles. Determining a standard area of the bait proved extremely difficult due to variability in size and shape of the particles. Baits were placed into appropriate size categories based on long-axis measurements, which may have affected the predictive ability of the regression. Median values for particle sizes removed were consistent with preferred sizes, as determined by bait removal and foraging. Observations made while selecting foraging ants 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 substantial mass removal of particle Sizes 4 (SGA) and 3 (CAS). Mean head capsule widths for ants foraging on both baits corresponded with head widths observed for monogyne workers, which were 0.88 ± 0.21 mm and 0.87 ± 0.37 mm for colonies from Georgia and Texas, respectively, (Greenberg *et al.* 1985). However, the presence of multiple wingless females in experimental colonies (possible polygyne queens) contradicts this. Greenberg *et al.* (1992) classified 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. What was apparent is that head capsule size could be used as an indicator of projected bait size preferences.

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