2005

Liquid feeding in the red imported fire ant, Solenopsis invicta Buren (Hymenoptera: Formicidae)

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LIQUID FEEDING IN THE RED IMPORTED FIRE ANT,
Solenopsis invicta Buren
(Hymenoptera: Formicidae)

A Thesis

Submitted to the Graduate Faculty of the
Louisiana State University and
Agricultural and Mechanical College
in partial fulfillment of the
requirements for the degree of
Master of Science

in

The Department of Entomology

by

Kathryn O'Brien
B.S.B.E., Louisiana State University, 2003
December 2005
ACKNOWLEDGMENTS

I thank Thabit Folami for her help with bioassays. I also thank Derek Dorman from the Department of Chemistry at LSU for his help with viscosity measurements. This work was supported by funding from the State of Louisiana Legislature special grant to L.M. Hooper-Bùi. I also acknowledge a scholarship from the National Conference on Urban Entomology in 2004. Lastly, I am greatly indebted to my major professor, Dr. Linda Hooper-Bùi, whose encouragement and support were vital in the completion of this work.
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ABSTRACT

In order to accurately test liquid bait effectiveness in the lab, we determined that a starvation time of 96h is more appropriate than 72h of starvation time for laboratory fire ants (Solenopsis invicta Buren) to better simulate foraging ants in the field. Densities and viscosities of two commercial baits and 20% sugar water at 25°C were measured then compared to amount of material consumed per ant at these physical properties. Mean densities of 20% sugar water, Dr. Moss, and Terro were 1.051, 1.287, and 1.354 g/mL, respectively, and viscosity of each bait treatment varied in the same order but more drastically (1.7, 32, 400 centipoise, respectively). Ants that feed on formulated baits exhibit feeding behaviors different from those which occur when feeding on sugar water. At first glance, one might conclude that the difference is due to the toxicant, but our findings suggest that physical properties of baits may be a factor in this change in feeding behavior.

In order to determine the effect of liquid physical properties on feeding, the method of liquid feeding was first determined. Next, sucrose solutions were prepared to test effects of viscosity and sucrose concentration on Solenopsis invicta separately. Solutions containing boric acid were also prepared to record the toxicant effect in these tests. Individual ants were offered a droplet of solution, then amount taken and time was recorded. Fire ants used suction to feed, whereas lapping movements of the glossa were not observed. Initial weight of ants explained about 40% of the variability in total crop load. Total crop load was found to depend on viscosity as well as initial ant weight. As viscosity increased in a 30% sucrose solution, relative crop load and intake rate decreased. According to these results, individual ants feeding from solutions without
boric acid will leave with a total crop load that is 54% dependent on individual motivation whereas the crop load of ants feeding from a 0.5% boric acid solution only 46% depended on individual motivation. One explanation may be that boric acid solutions in this study acted as unique food sources.
CHAPTER 1: OVERVIEW

Red imported fire ants (*Solenopsis invicta*) are a major pest in agricultural and urban settings. These ants are extremely aggressive, and an individual ant will sting a person multiple times while her sisters do the same. This invasive species originated from South America and has spread over a large part of southeastern United States. Recently, these ants have been found in Australia and China. One reason *S. invicta* is so successful is the ability of these ants to reproduce quickly (Vinson 1997). Another pest species, the Texas leafcutting ant *Atta texana* (Buckley), can defoliate trees; however, the reproduction rate of this species is slow (Walter 1938). Given the ability of *S. invicta* to spread so quickly, major efforts to rid areas of this species have been implemented.

One technique used to eradicate red imported fire ants is the dispersion of granular and liquid baits. Liquid baits, which take advantage of natural foraging habits of this insect, are available on the market for ant management; however, not much is known of *S. invicta* liquid feeding behavior. More specifically, does the insect lap liquid like a bumblebee, or does it suck liquid through a “straw” like a butterfly? The method of liquid feeding is important in liquid bait development, especially the viscosity and other physical properties of baits. Perhaps these ants cannot feed from highly viscous solutions, because its mouthparts cannot handle the resistance. Is sugar concentration a major factor in the final crop load of the ant? The answers to these questions may help to formulate more suitable liquid baits to control this pest.

In order to test these questions, methods needed to be developed to accurately measure behavior. For instance, how long should laboratory colonies be starved before bioassays are performed? This question was addressed by feeding commercial baits and
sucrose solutions to both ants from the field and laboratory ants starved at three and four days. This is a first step in understanding liquid feeding methods in red imported fire ants. The following objectives will be addressed:

1. Determine how long laboratory red imported fire ants should be starved to mirror behavior of ants from the field.
2. Examine and characterize mouthpart activities during feeding.
3. Quantify the effect of liquid viscosity and sucrose concentration on final crop load and intake rate of foraging *S. invicta* individuals.
4. Measure any differences in final crop load and intake rate when boric acid is added to solution.
CHAPTER 2: HUNGER IN RED IMPORTED FIRE ANTS AND THEIR BEHAVIORAL RESPONSE TO TWO LIQUID BAITS

2.1 Introduction

Red imported fire ants, *Solenopsis invicta* Buren, have been a major pest in southeast U. S. since the early twentieth century (Taber 2000), especially in and around homes, businesses, and anywhere there may be food or appropriate microhabitat. To help manage fire ants, several types of pest management systems have been developed, including baits. Baits have three main advantages: baits require very low concentrations of insecticide, they eliminate the need to treat the nest, and they provide suppression of the entire colony, instead of just the foraging workers (Klotz et al. 1997).

Three forms of baits used for the management of ants are granular, liquid, and gel. Granular baits have been developed and are used more readily because they are easy to use and maintain. Most just need to be distributed around mounds or broadcast only once for adequate suppression. Liquid and gel baits are also available, but little research has been performed on these even though they may work more efficiently than granular baits, especially in areas with little water availability (Vail et al. 2003). Red imported fire ants will collect liquid five times more frequently than solid food (Tennant and Porter 1991); therefore, liquid bait may infiltrate the mound faster than granular bait. Also, Silverman and Roulston (2001) showed that *Linepithema humile* (Mayr) handled liquid sucrose solution more efficiently than the same solution given in a gelatin form. In contrast, Kidd and Apperson (1985) show that *S. invicta* recruitment to liquid soybean oil bait is slower than recruitment to granular soybean oil bait; however, the method of delivery of the liquid bait (vials fitted with cotton wicks) limited the surface area available to ants. The

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granular bait offered larger surface area, a major factor in foraging rates (Kidd and Apperson 1985). Given the importance of liquid food in the mound, bait provided in liquid form will attract many ants.

New liquid baits need to be tested for palatability and attractiveness to the target ants. Many times, researchers offer large colonies of ants the baits alone or with a choice test to determine attractiveness in the laboratory (Reid and Klotz 1992, Klotz and Reid 1993, Hooper-Bùi and Rust 2000). Similar experiments are performed to determine palatability in the field. It is possible that physical properties of the baits and the time that the ants are starved affect the results of those experiments.

The amount of time food is withheld differs in laboratory bait acceptance studies. Sorensen et al. (1981) performed a feeding study with fire ants that were starved for three to five days. Klotz et al. (1997) and Glunn et al. (1981) only starved fire ants for one day. In another study, fire ants were starved 36 h before a foraging test (Pranschke et al. 2003).

Similar to starvation, sucrose concentration will also affect consumption in ants. Josens et al. (1998) tested multiple sucrose concentrations and found that *Camponotus mus* will leave a diluted (5-15% w/w) sucrose source with partial crop loads after two days of starvation. They hypothesized that this behavior may be adaptive because the ant would leave the food source early to communicate with nestmates and perhaps find a better food source. This, however, would not explain why ants ingested the diluted source (low viscosity) at a low intake rate. In a later study by Josens and Roces (2000), crop load and differences in intake rate were found to depend on the nutritional conditions of the colony (feeding motivation). Another study which was conducted on *S. invicta* feeding on
radiolabeled sucrose solution showed that crop load depended on time starved (Howard and Tschinkel 1980). Given these results, *S. invicta* behavior and amount of food consumed may be expected to vary with time starved in bait preference studies.

In order to accurately test liquid and gel bait effectiveness in the lab, I determined the starvation time required for laboratory fire ants to simulate foraging ants in the field. I also wanted to determine if the size of the fire ant could be associated with the amount of bait consumed. I measured the density and viscosity of each bait at 25°C then compared the amount of liquid or gel consumed per ant to these physical properties. According to Cohen (2003), viscosity is the most important characteristic of diet texture, and is a neglected topic in discussions regarding insect diets and liquid baits. The research described in this paper will provide data on the physical properties of selected baits which may be used to predict the response of ants to different types of liquid baits.

**2.2 Materials and Methods**

2.2.1 **Physical Properties.** Two types of commercial liquid baits were tested, Terro Ant Killer (Senoret Chemical Company, St. Louis, MO) containing 5.4% borax and Dr. Moss’s Liquid Bait System (J.T. Eaton and Co., INC., Twinsburg, OH) containing 1% boric acid. I tested two serial numbers (1100A, 1200A) of Terro Ant Killer. Twenty percent sugar water (weight: weight) was used as an untreated control. I placed 1.5 ml of each treatment in a pre-weighed micro-centrifuge tube and weighed them. Weight of the contents was divided by volume to obtain the density in g per ml. The density measurement of each liquid was replicated three times. The viscosity of the sugar water and each bait was measured using a Brookfield digital viscometer (Brookfield...
Engineering Laboratories, Inc., Stroughton, MA) and values are reported in centipoises (cP = mPa⋅s).

2.2.2 Ant Collection. Fire ant colonies were collected from Baton Rouge, LA in February and April 2003. Colonies were taken to the lab and gradually flooded in large buckets to remove ants from the soil. One liter of water was poured into each colony every hour until the colony was floating for easy removal of ants from the soil. Each colony was placed in a large tray (580 mm x 350 mm x 90 mm) with Teflon (Dupont, Wilmington, DE) coated around the sides so ants could not escape. I provided each laboratory colony with “condos,” 140 mm diam petri dishes with moist plaster of paris on the bottom and a lid darkened with black permanent marker to minimize light disturbance; water and 20% sugar water in 25 mm diam vials covered with mesh; and frozen crickets ad libitum. Colonies were also sampled from the field in Baton Rouge in June to compare with laboratory ants as a control. About 30 ants were aspirated from each mound.

2.2.3 Bioassay. The purpose of this experiment was to determine the starvation time required for laboratory fire ants to simulate the levels of hunger among foraging fire ants in the field. About 100 ants each from 12 monogyne, laboratory colonies were starved for either 72 or 96 hours. Ants were collected outside of condos to improve the chance of collecting older workers/foragers. They were placed in a rectangular box (175 mm x 80 mm x 40 mm) coated with Teflon on the inside sides to prevent escape. The ants were given a small 35 mm diam condo and a 6 mm diam vial of water with cotton wick. Terro Ant Killer and Dr. Moss’s Liquid Bait System were used as treatments and 20% sugar water (w:w) was used as an untreated control.
Hooper-Bùi and Rust (2001) developed a method to measure toxicity of bait to individual ants. I modified their procedure to determine the acceptance of the bait to individual ants in the laboratory. Each ant was weighed individually to the nearest 0.1µg with a Sartorius AG Micro SC-2 scale (Goettingen, Germany) in a 6 mm polyethylene genitalia vial (Bioquip Products, Inc., Rancho Dominguez, CA). The ant was released into a 20 mm petri dish and given a drop of sugar water or one of two baits on which to feed. After observing the ant drink to satiation and walk away from the food source, it was placed back in the vial and weighed again. The difference between the initial weight and the final weight determined the amount of liquid consumed.

All the replicates could not be performed at the same time so the experiment was conducted by subjecting the ants to the same starvation period, and all baits and the sugar water control were tested during this time. Individual ants were removed from three different colonies, starved, weighed, and offered the bait. I repeated this again for a total of six colonies and again for each starvation period for a total of 12 laboratory colonies used in this project. Ants from the field that were used as “controls” were aspirated from six different mounds. These ants were not analyzed for form (monogyne or polygyne); the landscape in Baton Rouge holds a mosaic of the two forms. Again, ants were captured outside the nest to improve the chance of collecting foragers. These field ants were used in the bioassay within one hour after their capture to measure their bait consumption to represent the degree of hunger ants exhibit in the field. The field ant data created a standard to which I could compare our starved laboratory ant consumption. Colonies were considered replicates and individual ants from their respective colonies were considered subsamples. Only ants that fed were used in the analysis. I used the density of
each liquid to calculate the volume that each ant consumed and the term I used for this is crop load after Josen et al. (1998). If body size was associated with crop load, I standardized the measurements by dividing the body weight into the crop load calculating relative crop load in microliters per milligram (Josen et al. 1998).

2.2.4 Field Choice Test. All baits and sugar water controls were placed near active foraging trails of fire ants. Measured amounts of bait and sugar water were placed in 1.5 ml microcentrifuge tubes, and they were placed on their sides equidistant from the ant foraging trail. This allowed the ants to make a choice between the baits and untreated sugar water control. I counted foragers visiting each vial at 20 min. Vials were collected at 1 hour, weighed, and amount consumed was calculated. Ants trapped in vials after collection were counted for the number of ants visiting at 1 hour.

2.2.5 Statistical Analysis. The density of liquids was compared using a one-way analysis of variance (ANOVA), then differences were determined between the means using Tukey’s adjustment (SigmaStat SPSS 3.0 2003). I compared initial ant weights using a one-way ANOVA to determine if there were differences among the weights of ants for each starvation period and bait type. I used a regression to determine if the initial weight of the ant could be used to predict the amount of liquid the ant would consume. Regression was performed with weight of ant as the independent variable and amount consumed as the dependent variable for each starvation time and bait type (SigmaStat SPSS 3.0 2003). I categorized the ants that fed into three weight categories and used an ANCOVA model in PROC MIXED to determine if larger ants could discriminate between bait and control sugar water (SAS Institute 2002).
I tested for difference in acceptance between the batches (based on serial numbers) of Terro (t-test). To determine if the ants differentially fed on the baits and sugar water control, I analyzed the data with a two-way ANOVA model. I transformed the data with a natural log (ln) transformation to meet the requirements of the parametric test (SigmaStat, SPSS 3.0 2003). I used the number of hours starved, 72 and 96, as treatments and field collected ants to test for differences; I also compared mean amount of bait consumed with 20% sugar water. I tested for interactions between time starved and amount of each bait consumed. For data with significant regressions of ant weight and crop load, I analyzed the relative crop load for each time period using one-way ANOVA (SigmaStat SPSS 3.0 2003). Means were separated by a Tukey adjustment with $\alpha<0.05$.

A one-way ANOVA was performed to determine if there were differences in amount of each bait taken when compared to the control. Significant means were separated by a Tukey adjustment with $\alpha<0.05$. I regressed the number of individuals at the bait at 20 minutes and one hour with the amount removed using linear regression (SigmaStat SPSS 3.0 2003). Lastly, I performed a repeated measures analysis using PROC MIXED (type = autoregressive(1)) on log plus one transformed data to see if the number of ants visiting at 20 and 60 minutes was different for each bait (SAS Institute 2002). Sample size requirements were met for the repeated measures analysis according to Von Ende (2001).

2.3 Results

2.3.1 Physical Properties. Density and viscosity were found to differ among treatments. The densities of 20% sugar water, Dr. Moss, and Terro were (mean ± SEM)
1.051 ± 0.004 g/mL, 1.287 ± 0.010 g/mL, and 1.354 ± 0.006 g/mL, respectively (Table 2.1). Significant differences were found between all density values (F = 470.9, df = 2, P < 0.001). The viscosity of each bait treatment varied in the same order (sugar water < Dr. Moss < Terro) but more drastically (1.7, 32, 400 cP).

Table 2.1. Density and viscosity of tested baits. Density was calculated by dividing the measured mass by a known volume of a sample of bait. Viscosity was measured using a digital viscometer. Means with different letters are significantly different (P < 0.05, Tukey adjustment).

<table>
<thead>
<tr>
<th>Bait</th>
<th>Density (g/mL)</th>
<th>Viscosity (cP)</th>
</tr>
</thead>
<tbody>
<tr>
<td>20% Sugar Water</td>
<td>1.051 ± 0.004 a</td>
<td>1.7</td>
</tr>
<tr>
<td>Dr. Moss</td>
<td>1.287 ± 0.010 b</td>
<td>32</td>
</tr>
<tr>
<td>Terro</td>
<td>1.354 ± 0.006 c</td>
<td>400</td>
</tr>
</tbody>
</table>

2.3.2 Size. The initial size of ants did not depend on whether they were from a lab colony or from the field; however, the decision to feed at a food source depended on the type of food. The initial weights of laboratory ants starved for 72 and 96 hours were similar (mean ± SEM, 0.824 ± 0.138 mg and 0.824 ± 0.129 mg, respectively) whereas ants from the field initially weighed more (mean ± SEM 1.20 ± 0.123 mg) but not significantly (F = 2.91, df = 2, P = 0.060). Sixty-six percent of ants (or 34 ants, n = 51) fed after 72 hours of starvation, 61% (or 24 ants, n = 39) fed after 96 hours of starvation, and 45% (or 33 ants, n = 72) field ants fed. Thirty-five percent (or 15 ants) of all ants offered Dr. Moss were observed to feed compared to 62% (or 46 ants) and 66% (or 30 ants) of ants offered Terro and sugar water, respectively (Figure 2.1). Grouped by bait treatment types, ants feeding on sugar water, Dr. Moss, and Terro initially weighed 0.861
± 0.133 mg, 0.935 ± 0.1912 mg, and 1.040 ± 0.110 mg (mean ± SEM), respectively, and were not significantly different (F = 0.47, df = 3, P = 0.702).

The association of initial ant weight and amount consumed depended upon whether the ants came directly from the field and how long the laboratory ants were starved. Initial weight of ants from the field was associated with amount of liquid (both baits and sugar water) consumed ($R^2 = 0.34$, $F = 16.2$, $P < 0.001$). Laboratory ants that were starved for 96 hours also exhibited an association of initial weight with amount of liquid consumed ($R^2 = 0.54$, $F = 34.5$, $P < 0.001$) but those that were starved for 72 hours did not ($R^2 = 0.01$, $F = 0.31$, $P = 0.58$).

**Figure 2.1.** The percentage of *S. invicta* that were observed to feed on the offered bait. The number of ants observed to feed for Dr. Moss, Terro, and sugar water was 15, 46, and 30, respectively.
Initial weight of fire ants can be used as a predictor of how much sugar water they will consume in the laboratory. When all baits and the control are considered, initial weight of all the ants in the study was significantly associated with consumption of bait ($R^2 = 0.29$, $F = 35.9$, $P < 0.001$). Sugar water consumption (mass) was significantly associated with initial ant weight ($R^2 = 0.54$, $F = 95.5$, $P < 0.001$; Figure 2.2), but both Dr. Moss and Terro consumption by ants were not associated with initial ant weight ($R^2 = 0.26$, $F = 4.61$, $P = 0.051$ and $R^2 = 0.053$, $F = 2.45$, $P = 0.12$, respectively). When I categorized sugar water control ants as small (0-0.61mg), medium (0.62-1.0 mg) and large (1.01-4.3 mg), only the weight of ants that were in the medium and large categories was associated with amount consumed ($t = 2.31$, $P = 0.02$; $t = 7.38$, $P < 0.0001$, respectively). The initial weight of small ants was not associated with amount consumed.

Figure 2.2. A plot of initial weight versus sugar water consumed. Linear regression represented, $R^2 = 0.54$, $P < 0.001$. 
(t = 1.01, P = 0.31). When all ants were analyzed together only the weight of the large ants was significantly associated with amount of sugar water consumed (t = 4.72, P < 0.0001).

2.3.3 Baits. No significant difference was found between the amount consumed for each starvation period/bait combination (F = 1.892, df = 6, P = 0.092). The degree of hunger in ants affected their consumption of the baits (F = 4.226, df = 2, P = 0.018; Figure 2.3). When the amount consumed by starved and field ants offered all bait types was compared, no significant difference was found between those starved for 96 hours and ants from the field (P = 0.987). Significant differences were found between both 96 hours and 72 hours (P = 0.027), and 72 hours and zero hours (P = 0.05).

![Figure 2.3](image_url)

**Figure 2.3.** The period of starvation as categories and the amount consumed for all baits. N, sample size; different letters are significantly different (P < 0.05).

The amount consumed was also affected by bait treatment type (F = 8.073, df = 3, P < 0.001). There were no differences (P = 0.983) between the batches (based on serial numbers) of Terro, therefore all the data for Terro were combined. Ants fed on sugar
water (0.242 mg ± 0.04; Mean ± SEM) more readily than Terro (0.081 mg ± 0.05; Mean ± SEM) and Dr. Moss (0.112 mg ± 0.07; Mean ± SEM). Significant differences were found between the amount consumed for sugar water and Terro (P = 0.002) and sugar water and Dr. Moss (P = 0.008); however, the amounts of Terro and Dr. Moss consumed were not different (P = 0.993). For ants that fed on Terro and Dr. Moss, comparison of the means between starvation groups shows no significant difference between 96 hour group and ants from the field (Figure 2.4). The mean amount consumed for the 72 hour group was less than the amount consumed for the 96 hour group and ants from the field for both baits and sugar water. Terro contains 5.4% borax (which is equivalent to 3.6% boric acid; Anonymous 1997) while Dr. Moss contains only 1% boric acid. These percentages (3.6% for Terro) were multiplied by the amount consumed for each bait and starvation period to obtain the total amount of toxicant consumed for each combination (Figure 2.4). Ants that fed on Dr. Moss consumed 0.251, 1.547, and 1.563 µg of toxicant, and those that fed on Terro consumed 1.646, 2.88, and 3.275 µg of toxicant after 72, 96, and 0 hours starvation (field ants), respectively.

The amount taken, after conversion from mass to volume (crop load), changed differentially as a consequence of the density of each bait (Table 1). The volumes of bait (mean ± SEM) ants removed were 0.231 ± 0.04, 0.06 ± 0.05, and 0.087 ± 0.06 of sugar water, Terro, and Dr. Moss, respectively. When volume was used as the dependant variable in a 2-way ANOVA analysis, the patterns of significance did not change; although, when comparing bait types the differences of means according to the Tukey adjustment were larger. If only comparing the starvation groups, conversion to volume is not necessary. When I analyzed relative crop load for sugar water ants (ants feeding on
sugar water had a final crop load that was significantly related to the initial weight), significant differences in amount removed were not observed ($F = 1.997$, $df = 2$, $P = 0.155$).

![Figure 2.4](image_url)

**Figure 2.4.** The average amount consumed for each bait and starvation period in bar graph; the toxicant consumed represented in insert.

### 2.3.4 Field Test

In the field choice test, the mean ($\pm$ SEM) amounts of sugar water, Terro, and Dr. Moss consumed were $0.182 \pm 0.06$ g, $0.104 \pm 0.03$ g, and $0.058 \pm 0.01$ g, respectively. No significant differences were found between amount consumed and liquid type ($F = 2.913$, $df = 2$, $P = 0.082$). The number of individuals visiting sugar water, Terro, and Dr. Moss vials were $7 \pm 3.61$ (mean $\pm$ SEM), $2 \pm 1.36$, and $0.286 \pm$
0.18 ants at 20 min and 42.14 ± 16.5, 21.86 ± 10.7, and 9.71 ± 6.5 ants at 60 min, respectively. Significant differences were found between the number of ants at 20 and 60 minutes at each bait type (F = 19.15, df = 1, P = 0.0072), but not among the baits (F = 2.55, df = 2, P = 0.114). However, when individual bait types were examined, correlations exist between the number of ants counted at vials and amount consumed. A significant relationship was found between the number of ants visiting sugar water vials at 20 minutes and amount consumed (R² = 0.971, F = 101, P = 0.002), but the number of ants visiting Dr. Moss and Terro vials at 20 minutes was not related to amount consumed (R² = 0.107, F = 0.480, P = 0.526; R² = 0.541, F = 4.718, P = 0.096). At one hour, both baits and sugar water were significantly correlated with amount consumed (sugar water: R² = 0.887, F = 31.4, P = 0.005; Dr. Moss: R² = 0.757, F = 15.6, P = 0.011; Terro: R² = 0.967, F = 145, P < 0.001; Figure 2.5).

2.4 Discussion

Our results indicate that 96 hours of withholding food for *S. invicta* leads to foraging behavior which more closely matches that observed from field ants when compared to only 72 hours of starvation. However, when starvation levels are compared in the sugar water group, the amount consumed was similar between 72 and 96 hour groups. One reason for this interaction is the laboratory ants were accustomed to feeding on sugar water while the field ants reacted differently to the unique sucrose food source. The major differences between the 72 and 96 hour groups are seen when comparing the commercial bait types. The mean differences in the amount consumed between Terro and Dr. Moss baits for each starvation period were heavily dependent on the acceptability of the baits. Almost two-thirds of the ants offered Terro accepted the bait compared to over
one-third of the ants offered Dr. Moss. This is also evident in the field choice test in which a more natural foraging pattern is seen for sugar water compared to the other bait types. Acceptability may be affected by the toxicant and the physical properties of the baits discussed below.

![Graph showing the number of ants counted at vials after 60 min and the total amount consumed after 60 min for all three baits.](image)

**Figure 2.5.** The number of ants counted at vials after 60 min and the total amount consumed after 60 min for all three baits. Sugar water, $R^2 = 0.967$, $P < 0.001$; Terro, $R^2 = 0.967$, $P < 0.001$; Dr. Moss, $R^2 = 0.757$, $P = 0.011$.

A significant relationship was shown for initial fire ant weight and amount of sugar water consumed. This relationship was highly significant ($P < 0.001$), which has also been found with the carpenter ant, *Camponotus mus* (Josens et al., 1998). However, when ants fed on Terro and Dr. Moss, a significant relationship between ant weight and
bait consumed was not found. There are two possibilities for this change in behavior: (1) the insecticide in each commercial ant bait may produce a negative behavioral reaction, such as avoidance or irritation, and interrupt the normal feeding pattern of an individual (Ave, 1995), or (2) the viscosity of the bait changes foraging behavior (discussed later). Hooper-Bùi and Rust (2000) suggest that Argentine ant feeding behavior changes as concentration of boric acid increases. While the total amounts of Terro and Dr. Moss consumed were not significantly different, the toxicant load of ants that fed on Terro was higher than for those that fed on Dr. Moss, especially for ants starved 96 hours and ants from the field. Changes in feeding behavior with increasing toxicant concentration were shown previously in *S. invicta*. Klotz et al (1997) fed multiple concentrations of boric acid in 10% sugar water solution to *S. invicta*. All solutions would have had the same viscosity which eliminates it as a possible cause of varying ant behavior. At 5% and 1% boric acid, consumption was reduced compared to the 10% sugar water control.

Ants offered Terro ate 62% of the time, about the same as sugar water. Knowing that only 35% of ants offered Dr. Moss in the bioassay consumed some bait, avoidance or irritation may have occurred due to the Dr. Moss bait matrix. On the other hand, Terro may not cause negative effects on the behavior of red imported fire ants until after some portion is consumed. It is possible that the differences in consumption that I measured are not due to concentration of toxicant or viscosity of the bait but to the individual hunger of the foraging ant. Future experiments will tease out these details.

Physical properties were different among the two baits and the sugar water control. In addition to the effects of a toxicant, Terro also has the negative effects of viscosity on intake rate, which may explain why the amount consumed for Terro was less
than sugar water in the time allotted in the field test. According to the number of ants that fed on each bait in the laboratory, Dr. Moss is less attractive than Terro or 20% sugar water, which explains why the Dr. Moss bait was least preferred in the field choice test, irrespective of the viscosity. Baits that are more dense have more mass per volume and may cause the ant to cease feeding with a lower crop load than when they feed on sugar water for all starvation times. As with diluted solutions, Josen et al. (1998) found that *Camponotus mus* ants will leave a concentrated (70%) sucrose source with partial crop loads. They proposed that, as sucrose concentrations increased past 15%, the final crop load was dependent on the physical properties of the solution such as viscosity and density.

Along with mass values, I also analyzed the data using units of volume. Because all of the densities were greater than one, volume values were less than mass values. The difference between mass and volume values varied with the type of bait treatment. So, mean differences and P-values of crop load will be different than those associated with mass when compared between bait types but will be the same when compared between starvation times. For example, the mean mass of Dr. Moss bait taken is 0.112 mg, and the mean volume of Dr. Moss taken is 0.087 µL. The amount of sugar water taken is 0.242 mg or 0.231 µL. It is important to understand which measurement is appropriate for the question one is trying to answer. When I compared the differences in starvation, the crop load was irrelevant and need not be calculated, and when considering differences in consumption between bait types, crop load could be crucial.

The reason I analyzed the crop load was to calculate the relative crop load for sugar water fed ants. When I analyzed the relative crop load, I may have negated the
nutritional state of the colony. That is, ants that were starved for 96 hours may weigh less than similar ants that were starved for a shorter time period. This may explain why no significant differences were found in the relative crop load between the starvation times of sugar water fed ants.

In conclusion, ants that feed on formulated baits exhibit feeding behaviors different from those which occur when feeding on sugar water. At first glance, one might conclude that it is the presence of the toxicant, but our findings on the physical properties of the baits indicate that they may be a factor in this change. When concentration of toxicant for liquid baits are investigated initially, the effective dose may be investigated with sugar water prior to formulation. The final formulation of the liquid bait may be vastly different from that which was tested and the physical properties of the bait may be a factor in ant acceptance of the bait. I highlight these differences in ant behavior; however, more research is needed to further describe the effects of viscosity and toxicants on *S. invicta* at liquid baits.
CHAPTER 3: FEEDING IN S. INVICTA: EFFECTS OF SUCROSE CONCENTRATION, VISCOSITY, AND BORIC ACID ON TOTAL CROP LOAD AND INTAKE RATE

3.1 Introduction

The mechanics of liquid feeding in nectarivorous insects have been studied extensively in honeybees (Schmid-Hempel et al. 1985, Farina and Nunez 1995, Tezze and Farina 1999), bumblebees (Harder 1986), Lepidoptera (Kingsolver and Daniel 1979, May 1985, Josens and Farina 1997, 2001), and ants (Josens et al. 1998, Paul et al. 2002, Paul and Roces 2003). In ants, the choice of a food source and/or amount of food taken has been explained by variables such as available surface area (Wilson 1962, Silverman and Roulston 2001), distance of food source from nest (Taylor 1977), and level of starvation (Howard and Tschinkel 1980, 1981, Josens and Roces 2000). The morphological characteristics of the insect’s mouthparts and the physical properties of the solution, along with the pressure difference created by the insect while feeding, also may influence the dynamics of liquid intake (Kingsolver and Daniel 1979).

Two general liquid-feeding techniques are employed by insects: suction and/or lapping. Some insects, such as butterflies, will use their long proboscis as a straw to suck nectar or other liquids (Kingsolver and Daniel 1995). Others, such as bumblebees and honeybees, use their glossa as a lapping shovel or sponge to rhythmically extend and retract it into and out of the liquid food source (Harder 1986). Whether mouthparts are used for suction or lapping, the force that brings liquid into the food canal is generated by compression and dilation of the cibarium, so called the cibarial pump (Josens and Roces 2000). In the case of ants, Paul and Roces (2003) found that all 11 species tested could employ both techniques; nevertheless, each species preferred one over the other. One ant
species, *Pachycondyla villosa* (Fabricius), licked the solution and transported it as a droplet held between its mandibles. Another species, the carpenter ant *Camponotus mus* Roger, will use its glossa as an open passive duct to suck a large droplet of liquid to be stored in the crop inside the gaster, then will switch to lapping when the droplet decreases to a thin film. This behavior has also been observed in honeybees (Josens and Roces 2000).

Besides the characteristics of the insect’s feeding apparatus, other important factors that influence liquid feeding are physical properties of the liquid solution. These may include the concentration of a carbohydrate, amino acid, toxicant, viscosity, density, or surface tension, etc. Sucrose solutions are often used to investigate variables such as optimal sugar concentration for solution and energy (sucrose) intake rate, fastest rate of solution and energy intake, and crop load, which all may vary greatly depending on the species. For example, butterflies maximize their energy intake with sucrose concentrations of 35-40% (w/w) (May 1985) whereas rate of energy intake in the bumblebee and honeybee is fastest at 50-65% sucrose solutions (Harder 1986). In contrast, Josens *et al.* (1998) found that *C. mus* ants (a species that uses suction when ingesting liquids) maximize energy intake with 41.5% sucrose, similar to butterflies.

Energy intake is one similarity this carpenter ant species has with butterflies. In the case of solution intake rates, butterflies (Josens and Farina 1997, 2001) and bees (Harder 1986) intake solution at constant rates at the maximum value for diluted solutions up to a critical sucrose concentration which is specific to each species. At the critical concentration value, solution intake rates will begin to decline because viscosity begins to increase exponentially at 50% sucrose, making it difficult for the insect to
produce the same pressure drop in the cibarial pump. Hereafter, “critical” values refer to the sucrose concentration (or viscosity) at which intake rates (or another response variable) start to decrease, increase, or stay constant. Under these conditions, viscosity should be the only obstacle that keeps an insect from ingesting a dilute solution at the fastest rate possible. This has also been found in another carpenter ant, *C. rufipes*, which is also observed to use suction as its preferred liquid-feeding technique (Paul and Roces 2003).

In contrast, a peculiar behavior has been found in the aforementioned carpenter ant species, *C. mus*, with respect to solution intake rates. Instead of ingesting diluted solutions at the maximum rate, *C. mus* individuals increased intake rates up to a critical value of 30.8% sucrose (Josens *et al.* 1998). In a later study by Josens and Roces (2000), they suggest this behavior of ingesting diluted solutions at lower intake rates may be caused by the nutritional state of the colony. In other words, solution intake rates for some species depend not only on the physical properties of the solution and the characteristics of the feeding apparatus, but also on the feeding motivation of the individual. Honeybees and ants have also been found to leave a diluted sucrose food source with partial crop loads (Josens *et al.* 1998). One theory is the insects leave the unprofitable food source faster in order to maintain the link with the colony to gather information for more rewarding food sources (Nunez 1982, Schmid-Hempel *et al.* 1985).

Viscosity increases as sucrose concentration increases. Until recently, studies have not quantified the effects of sucrose concentration and viscosity separately. By isolating these two variables, we may identify the exact mechanisms that control feeding responses in ants. For instance, it may be a purely chemosensory process in which an
ant’s individual feeding motivation controls feeding behavior or a mechanical one in which viscosity alone controls intake rate and/or crop load. Both factors are likely to play a role in feeding behavior, but how important are they? Farina and Josens (1994) utilized a method that allows these two variables to be tested, and this method has been applied on honeybees and moths and is discussed below.

Many experiments have been conducted to explain trophallaxis behavior in honeybees. Farina and Nunez (1995) found the total volume exchanged between donor and recipient honeybees depends on the amount carried in the crop of the donor bee. The total volume exchanged does not depend on the sugar concentration or the viscosity of the solution. However, the transfer rate of solution depends on both. When viscosity was held constant, the transfer rate of solution was constant up to 30% sucrose then increased with more concentrated solutions. When sucrose concentration was held constant, this rate declined as viscosity increased to a critical viscosity of 0.12 cm$^2$/s then leveled out (Tezze and Farina 1999). Compare this critical viscosity value to that associated with the solution intake rate (note: not transfer rate) of the moth Macroglossum stellatarum; the intake rate continued to decline as viscosity increased up to 0.35 cm$^2$/s then leveled out. In this insect, intake rate was constant then declined with concentrated solutions higher than 30% sucrose when viscosity was held constant (Josens and Farina 2001). In both of these insects, sucrose concentration and viscosity played a part in transfer/intake rate.

One goal of my research is to observe the feeding technique(s) of S. invicta and determine whether the behavior more closely resembles that of the butterfly (suction) or the bee (lapping). Once I am able to characterize the red imported fire ant’s feeding technique, I can accurately describe the results I obtain from my other experiments with
viscosity and sugar and boric acid concentration. I will measure intake rate and crop load in constant viscosity and constant sucrose concentration experiments. Also, it is well documented that toxicants such as boric acid negatively affect bait consumption in ants (*S. invicta*: Klotz et al. 1997; *Linepithema humile*: Klotz et al. 1998), but it is unknown whether it directly affects feeding behavior or recruitment behavior. To observe the effects of boric acid on feeding behavior while sucrose concentration and viscosity are held constant, the procedures will be run a second time with boric acid added to each solution. These results may offer explanations as to how *S. invicta* behavior changes when offered liquid bait.

3.2 Materials and Methods

3.2.1 Liquid Feeding Behavior. Individual ants were observed under an Olympus OLY-750 microscope. Twenty percent sucrose solution was offered as a droplet and as a thin film to an ant (Paul and Roces 2003). Feeding behaviors such as mode of liquid intake, movement of mandibles, and movement of glossa were noted.

3.2.2 Solution Series. Various amounts of Tylose (Clariant International Ltd., Charlotte, NC) mixed with sucrose-water solutions were used to develop six series of solutions in which viscosity and sucrose concentration effects were evaluated separately (Farina and Josens 1994, Tezze and Farina 1999; Josens and Farina 2001). The small amounts of Tylose needed to increase the viscosity of a solution did not significantly change its density (Farina and Josens 1994). The first series is the base series (BS) in which six sucrose concentrations, 10, 20, 30, 40, 50, and 60% (w/w), were offered to individual, pre-weighed ants. A constant concentration series (CCS) was prepared by adding varying amounts of Tylose (0.1, 0.2, 0.3% w/w) to a 30% (w/w) sucrose solution.
Therefore, solutions in this series had a sucrose concentration of 30% (w/w) and differing viscosities (0.749, 0.1863, 0.3552 cm$^2$/s, respectively) corresponding to more concentrated sucrose solutions. The constant viscosity series (CVS) was 10, 20, 30, 40, and 50% (w/w) sucrose solutions mixed with Tylose (0.253, 0.208, 0.150, 0.080% w/w, respectively) to produce a viscosity (0.125 cm$^2$/s) that corresponds to a 50% (w/w) sucrose solution (Farina and Josens 1994). The last three series were prepared as above except each sucrose solution contained 0.5% (w/w) boric acid (Klotz et al. 1997). BS solutions with boric acid added were termed BA, CVS solutions with boric acid added are BCVS, and CCS solutions with boric acid are BCCS. Solutions referred to as “constant concentration tests” are the CCS and BCCS whereas those referred to as “constant viscosity tests” are the BS, CVS, BA, and BCVS series. Data from the BS (controls) were collected with each corresponding treatment series. Preliminary tests were performed to assure that Tylose could not be detected by the ants. Ants consumed solutions which contained the highest concentrations of Tylose as eagerly as ants that consumed 20% sugar water.

3.2.3 Bioassay. Five monogyne colonies of *S. invicta* were collected from Baton Rouge, LA. Each series of solutions was tested with individual foragers from these five colonies (=replicates). Large trays (580 mm x 350 mm x 90 mm) with Teflon-coated (Dupont, Wilmington, De) sides were used to house each colony. Before tests began and between tests, ants were fed 20% (w/w) sugar water, frozen crickets, and water *ad libitum*.

Bioassays were performed in the same manner for each series of solutions. Colonies were starved for 96 hours to achieve uniform hunger before tests began.
(O’Brien and Hooper-Bùi in press). One at a time, colonies were connected to a smaller foraging arena by a wooden bridge where a droplet of sucrose solution was presented (Josens et al. 1998). A small group of foragers were permitted access to the arena and allowed to feed. After the foragers were allowed back to the colony to presumably recruit others, single ants were allowed on the bridge and weighed (in mg) by capturing the ant in a 6mm genitalia vial. All weights were recorded to the nearest 0.1µg with a Sartorius Micro SC-2 scale. The ant was placed back on the bridge to continue to the foraging arena, or the ant may be placed directly in the arena. The feeding time was measured as the total time the ant was in contact with the droplet. After feeding, the ant was captured once again on the way back to the colony via the wooden bridge and weighed. Data from at least 15 ants (=subsamples) from each treatment combination were obtained.

3.2.4 Viscosity Measurements. Viscosity measurements of boric acid solutions (0, 0.5, 1, and 5% boric acid) in 20% sugar water were obtained to determine if boric acid changes viscosity of solutions. A Cannon-Ubbelohde viscometer (size 200) was used for measurements. All experiments were completed at room temperature (~25C); therefore, viscosities were measured in the same conditions. Three samples of each solution were prepared and measured for viscosity. Viscosities are presented as kinematic viscosities expressed in cm²/s.

3.2.5 Calculations and Statistical Analysis. The amount taken (mg) is the final weight minus the initial weight, and the volume taken (crop load, in µl) is the amount taken divided by the density of the solution obtained from tables (Lide 2002). Relative crop load (µl/mg) was calculated as crop load divided by initial ant weight. The CORR procedure was used to confirm the evidence of a correlation of crop load and initial ant
weight exists (SAS Institute 2002). The intake rate ($\mu l/min$) is the crop load divided by feeding time. Sucrose intake rate ($\mu g/sec$) is the intake rate multiplied by the concentration of sucrose in each sample.

Three dependent variables (relative crop load, intake rate, and sucrose intake rate) were analyzed separately in two-way ANOVA models. Series and sucrose/tylose concentration were the independent factors used to describe the two dependent variables. A total of five analyses were performed using PROC MIXED: (1) relative crop load, (2) intake rate, and (3) sucrose intake rate analyzed in the constant viscosity tests with four levels of series (BS, CVS, BA, and BCVS) and five levels of sucrose concentration (10, 20, 30, 40, and 50% sucrose); (4) relative crop load and (5) intake rate analyzed in the constant concentration tests with two levels of series (CCS and BCCS) and four levels of tylose concentration (0, 0.1, 0.2, and 0.3% tylose). A log transform was performed on all dependent variables to confirm the evidence of normal data (Shapiro-Wilks normality test, $P>0.05$). Tukey post-hoc tests were used in all analyses with $\alpha=0.05$ to determine differences between means. The GLM procedure was used to determine how much variability viscosity accounts for total crop load in the constant concentration tests by using a one-way ANOVA model. Lastly, two-way ANOVA was used to account for variability of total crop load with both initial weight and viscosity as predictors. Crop load was transformed to satisfy the normality assumption in ANOVA by a square root transformation (Shapiro-Wilks normality test, $P>0.05$, SAS Institute 2002).

### 3.3 Results

Under magnification, *S. invicta* individuals consumed 20% sucrose solution. Mouthparts extended into the droplet and remained motionless. Lapping movements of
the glossa were not observed. A thin film of solution was also offered to individuals; ants were not observed to drink when offered solution this way.

Significant correlations were found between initial weight of the ant and the volume of solution consumed for both sets of tests (Figure 3.1a and 3.1b). Average initial weights of individuals for the five colonies were 505.2 ± 26.06µg, 525.7 ± 24.69µg, 877.3 ± 78.24µg, 734.8 ± 51.52µg, and 836.2 ± 53.55µg, respectively. In the constant concentration tests, the correlation coefficient was 0.6318 (P<0.0001, Figure 3.1a) and was 0.5201 (P<0.0001, Figure 3.1b) with total crop load in the constant viscosity tests. Given this significant correlation between initial weight and volume consumed, relative crop load was calculated and used to compare volume of solution taken between individuals that are of different size, because red imported fire ants are a polymorphic species. Also, total crop load was found to significantly depend on viscosity in constant concentration tests for both the CCS (P = 0.0019, r² = 0.146) and BCCS (P < 0.0001, r² = 0.406). When both initial weight and viscosity are used as predictors of total crop load, less variability in the CCS is accounted for (P < 0.0001, r² = 0.457) than in the BCCS (P < 0.0001, r² = 0.542).

Figure 2 illustrates the relative crop load in constant concentration (Figure 3.2a) and constant viscosity tests (Figures 3.2b and 3.2c, averages of all variables in Table 3.1 for constant concentration tests and Table 3.2 lists constant viscosity tests). Significant differences were found among series in the constant viscosity tests (BS, CVS, BA, BCVS; F=17.72, df=3, P<0.0001); however, when individual series were compared, solutions and their corresponding boric acid solutions (example: BS and BA) were not different when relative crop load was compared (BS v. BA: P=0.3144; CVS v. BCVS: ...)
Figure 3.1. Initial weight and amount consumed by each ant in the constant concentration tests (a) and constant viscosity tests (b). Correlation coefficient for the constant concentration tests is $r = 0.6318$ ($P < 0.0001$) and for the constant viscosity tests is $r = 0.5201$ ($P < 0.0001$).
Figure 3.2. Relative crop load in constant concentration (CCS and BCCS) tests (a) in which all solutions include 30% sucrose and constant viscosity tests (b, c) in which constant viscosity series (CVS) and constant viscosity series with 0.5% boric acid (BCVS) include solutions with a viscosity of 0.125cm²/s, corresponding to a 50% sucrose solution. Error bars represent standard error, and crop loads that are significantly different are expressed by different letters according to Tukey analysis ($\alpha = 0.05$). Relative crop load = volume of solution consumed (µl)/initial mass of ant (mg); sample sizes for the base series (BS): 21, 34, 46, 23, 22, constant viscosity series (CVS): 13, 13, 10, 11, 20, base series with boric acid (BA): 14, 15, 14, 14, 16, constant viscosity series with boric acid (BCVS): 13, 16, 11, 13, 15 for 10, 20, 30, 40, and 50% sucrose, respectively; constant concentration series (CCS): 55, 14, 15, 15, constant concentration series with boric acid (BCCS): 14, 12, 14, 14 for 0.0287, 0.0749, 0.1863, 0.3552cm²/s viscosities.
P=0.9729). Similarly, there was no significant difference between the two series in the constant concentration tests (F=1.01, df=1, P=0.3164); therefore, boric acid solutions will not be mentioned in further relative crop load results. In the CCS, relative crop load decreases from $0.1653 \pm 0.01628 \mu l/mg$ at $0.0287 \text{cm}^2$/sec (solution with no tylose) to $0.0363 \pm 0.01026 \mu l/mg$ (mean ± SEM) at a critical viscosity somewhere near $0.3552 \text{cm}^2$/sec (0.3% Tylose solution; Figure 3.2a) which corresponds to almost a five-fold decrease.

**Table 3.1** Viscosity, relative crop load, and solution intake rate for solutions in the constant concentration series (CCS) and constant concentrations series with boric acid (BCCS). All solutions contain 30% sucrose, and the BCCS also contains 0.5% boric acid.

<table>
<thead>
<tr>
<th>Series</th>
<th>Viscosity (g/cm$^3$)$^a$</th>
<th>Relative Crop Load (µl/mg)$^b$</th>
<th>Solution Intake Rate (µl/min)$^b$</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCS</td>
<td>0.0287</td>
<td>0.16 ± 0.016</td>
<td>0.039 ± 0.0030</td>
</tr>
<tr>
<td></td>
<td>0.0749</td>
<td>0.13 ± 0.024</td>
<td>0.025 ± 0.0042</td>
</tr>
<tr>
<td></td>
<td>0.1863</td>
<td>0.095 ± 0.018</td>
<td>0.012 ± 0.0013</td>
</tr>
<tr>
<td></td>
<td>0.35525</td>
<td>0.036 ± 0.010</td>
<td>0.0075 ± 0.0017</td>
</tr>
<tr>
<td>BCCS</td>
<td>0.0287</td>
<td>0.16 ± 0.033</td>
<td>0.046 ± 0.0089</td>
</tr>
<tr>
<td></td>
<td>0.0749</td>
<td>0.14 ± 0.047</td>
<td>0.016 ± 0.0031</td>
</tr>
<tr>
<td></td>
<td>0.1863</td>
<td>0.031 ± 0.012</td>
<td>0.0058 ± 0.0017</td>
</tr>
<tr>
<td></td>
<td>0.35525</td>
<td>0.068 ± 0.021</td>
<td>0.0062 ± 0.0015</td>
</tr>
</tbody>
</table>

$^a$ Values from Josens and Farina (2001)

$^b$ Mean ± SEM
Table 3.2 Viscosity, average relative crop load, average solution intake rate, and average sucrose intake rate for each solution in the constant viscosity tests. Solutions in the CVS and BCVS series have a viscosity matching a 50% sucrose solution whereas solutions in the BA and BCVS series contain 0.5% boric acid. Statistics here are comparing series with no boric acid to those with boric acid (BS v. BA and CVS v. BCVS) within each variable (relative crop load, solution intake rate, sucrose intake rate).

<table>
<thead>
<tr>
<th>Series</th>
<th>Sucrose Concentration (%)</th>
<th>Viscosity (g/cm³)a</th>
<th>Relative Crop Load (µl/mg)b</th>
<th>Solution Intake Rate (µl/min)b</th>
<th>Sucrose Intake Rate (µg/min)b</th>
</tr>
</thead>
<tbody>
<tr>
<td>BS</td>
<td>10</td>
<td>0.013</td>
<td>0.23 ± 0.024 a</td>
<td>0.047 ± 0.005 a</td>
<td>0.079 ± 0.008 d</td>
</tr>
<tr>
<td>BS</td>
<td>20</td>
<td>0.019</td>
<td>0.13 ± 0.014 ab</td>
<td>0.041 ± 0.003 ab</td>
<td>0.14 ± 0.011 bc</td>
</tr>
<tr>
<td>BS</td>
<td>30</td>
<td>0.028</td>
<td>0.19 ± 0.017 a</td>
<td>0.038 ± 0.003 ab</td>
<td>0.19 ± 0.015 ab</td>
</tr>
<tr>
<td>BS</td>
<td>40</td>
<td>0.061</td>
<td>0.14 ± 0.018 ab</td>
<td>0.030 ± 0.003 b</td>
<td>0.20 ± 0.023 ab</td>
</tr>
<tr>
<td>BS</td>
<td>50</td>
<td>0.125</td>
<td>0.18 ± 0.026 a</td>
<td>0.030 ± 0.003 ab</td>
<td>0.25 ± 0.023 a</td>
</tr>
<tr>
<td>BA</td>
<td>10</td>
<td>0.013</td>
<td>0.20 ± 0.024 a</td>
<td>0.051 ± 0.006 ab</td>
<td>0.085 ± 0.010 cd</td>
</tr>
<tr>
<td>BA</td>
<td>20</td>
<td>0.019</td>
<td>0.19 ± 0.017 a</td>
<td>0.048 ± 0.004 ab</td>
<td>0.16 ± 0.013 ab</td>
</tr>
<tr>
<td>BA</td>
<td>30</td>
<td>0.028</td>
<td>0.16 ± 0.033 ab</td>
<td>0.056 ± 0.009 ab</td>
<td>0.28 ± 0.047 a</td>
</tr>
<tr>
<td>BA</td>
<td>40</td>
<td>0.061</td>
<td>0.079 ± 0.017 b</td>
<td>0.035 ± 0.003 ab</td>
<td>0.23 ± 0.023 ab</td>
</tr>
<tr>
<td>BA</td>
<td>50</td>
<td>0.125</td>
<td>0.17 ± 0.045 ab</td>
<td>0.033 ± 0.002 ab</td>
<td>0.27 ± 0.017 a</td>
</tr>
</tbody>
</table>

(Table 3.2 continued)
<table>
<thead>
<tr>
<th>Series</th>
<th>Sucrose Concentration (%)</th>
<th>Viscosity (g/cm³)</th>
<th>Relative Crop Load (µl/mg)</th>
<th>Solution Intake Rate (µl/min)</th>
<th>Sucrose Intake Rate (µg/min)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CVS</td>
<td>10</td>
<td>0.15 ± 0.040 abc</td>
<td>0.011 ± 0.002 cd</td>
<td>0.019 ± 0.003 f</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.077 ± 0.018 bc</td>
<td>0.011 ± 0.001 cd</td>
<td>0.036 ± 0.005 de</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.05 ± 0.013 bc</td>
<td>0.014 ± 0.001 cd</td>
<td>0.069 ± 0.006 bc</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.072 ± 0.012 abc</td>
<td>0.015 ± 0.003 cd</td>
<td>0.099 ± 0.017 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.19 ± 0.027 a</td>
<td>0.029 ± 0.003 ab</td>
<td>0.25 ± 0.024 a</td>
<td></td>
</tr>
<tr>
<td>BCVS</td>
<td>10</td>
<td>0.13 ± 0.028 abc</td>
<td>0.016 ± 0.003 bcd</td>
<td>0.027 ± 0.005 ef</td>
<td></td>
</tr>
<tr>
<td></td>
<td>20</td>
<td>0.12 ± 0.034 abc</td>
<td>0.019 ± 0.004 bc</td>
<td>0.064 ± 0.013 bcd</td>
<td></td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>0.072 ± 0.014 abc</td>
<td>0.0083 ± 0.002 d</td>
<td>0.042 ± 0.008 cde</td>
<td></td>
</tr>
<tr>
<td></td>
<td>40</td>
<td>0.071 ± 0.025 c</td>
<td>0.017 ± 0.003 cd</td>
<td>0.11 ± 0.022 b</td>
<td></td>
</tr>
<tr>
<td></td>
<td>50</td>
<td>0.18 ± 0.047 ab</td>
<td>0.033 ± 0.002 a</td>
<td>0.27 ± 0.017 a</td>
<td></td>
</tr>
</tbody>
</table>

- Values from Josens and Farina (2001)
- Mean ± SEM; means significantly different separated by different letters (Tukey analysis, α=0.05)
Unlike the CCS, relative crop load in constant viscosity tests showed differences within series (Figures 3.2b and 3.2c). Although, no significant differences were found in relative crop load which averaged 0.1741 ± 0.01986µl/mg (mean ± SEM) within the BS, differences were found within the CVS. Relative crop load in \textit{S. invicta} decreased (but not significantly) with increasing sucrose concentration in the CVS. However, at 50% sucrose (mean ± SEM; 0.1856 ± 0.02717µl/mg), relative crop load was more than twice that of 20% (0.07689 ± 0.01775µl/mg; \(P=0.0277\)) and more than triple that of 30% sucrose (0.0547 ± 0.01277µl/mg; \(P=0.0041\)) but not different from relative crop load at 10% sucrose (0.1501 ± 0.03959µl/mg; \(P=0.99\)).

Similar to relative crop load, differences in solution intake rate were found among series in constant viscosity tests (\(F=93.64, \text{df}=3, P<0.0001\), Figure 3.3b and 3.3c), but the addition of boric acid affected most intake rates of \textit{S. invicta}. Intake rates in the BS and BA were significantly different (\(P=0.0165\)), but were not in the CVS and BCVS (\(P=0.7831\)). Also, the intake rates of the CCS and BCCS were significantly different (\(F=6.95, \text{df}=1, P=0.0093\)). Intake rates in the CCS decreased with increasing viscosity from 0.03903 ± 0.002995µl/min at 0.0287cm²/sec to 0.007537 ± 0.001709µl/min (mean ± SEM) at 0.3552cm²/sec (Figure 3.3a) with a critical viscosity reached at around 0.25cm²/sec. The decrease in intake rate with increasing viscosity mentioned above follows closely with trends found in relative crop load also in constant concentration tests. Intake rates decreased from 0.04602 ± 0.008888µl/min to 0.006206 ± 0.001462µl/min at the same viscosities as above with a critical viscosity reached earlier around 0.15cm²/sec in the BCCS.
Figure 3.3. Intake rates in constant concentration (CCS and BCCS) tests (a) in which all solutions include 30% sucrose and constant viscosity tests (b, c) in which constant viscosity series (CVS) and constant viscosity series with 0.5% boric acid (BCVS) include solutions with a viscosity of 0.125 cm$^2$/s, corresponding to a 50% sucrose solution. Error bars represent standard error, and crop loads that are significantly different are expressed by different letters according to Tukey analysis ($\alpha = 0.05$). Intake rate = volume of solution consumed (µl)/feeding time (min).
Trends of intake rates found in the constant viscosity tests were very different from those found in the constant concentration tests. A significant decline in intake rates was observed in the BS (P=0.0439) as sucrose concentration increased from 10% (0.04748 ± 0.004592µl/min; mean ± SEM) to 40% sucrose (0.03023 ± 0.003417µl/min; Figure 3.3a). At 40% sucrose, intake rates plateaued. Boric acid seemed to have a phagostimulatory effect in the BA series in which the average intake rate was 0.03587 ± 0.002924µl/min (Figure 3b). In contrast to the BS, intake rates in the CVS stayed constant throughout except for the 50% solution (intake rate: 0.02947 ± 0.002838µl/min) which has no Tylose. The average intake rate in the CVS from 10% to 40% sucrose concentration was 0.0125 ± 0.0008780µl/min (mean ± SEM; Figures 3.3a and 3.3b).

Like the constant concentration tests, sucrose intake rates exhibited consistent trends (Figure 3.4). Significant differences were found between series (F=93.64, df=3, P<0.0001). Similar to intake rates in the constant viscosity tests, the BS and BA series were significantly different (P=0.0165). Over a three-fold increase was observed in BS sucrose intake rates as sucrose concentration increased from 10% (4.7479 ± 0.4592µg/min; mean ± SEM) to 50% sucrose (14.85 ± 1.358µg/min, Figure 3.4a); in the BA, rates were higher than the BS and increase similarly except at the 30% sucrose concentration solution. At this concentration, the average sucrose intake rate was much higher (16.65 ± 2.824µg/min; Figure 3.4b) than the corresponding solution in the BS (11.38 ± 0.8875µg/min) but not significantly (P=0.502). An increase in sucrose intake rates was also observed in the CVS, but with lower magnitudes similar to solution intake rates in CVS (Figure 3.4a).
Figure 3.4. Sucrose intake rates in constant viscosity tests (a, b) in which constant viscosity series (CVS) and constant viscosity series with 0.5% boric acid (BCVS) include solutions with a viscosity of 0.125cm$^2$/s, corresponding to a 50% sucrose solution. Error bars represent standard error, and crop loads that are significantly different are expressed by different letters according to Tukey analysis ($\alpha = 0.05$). Sucrose intake rate = intake rate (µl/min) * mass of sucrose in solution (µg).

Given the differences in intake rates between series with and without boric acid, viscosity measurements were taken to confirm viscosities did not change when boric acid is added. For 20% (w/w) sucrose solutions with 0, 0.5, 1, and 5% boric acid (w/w), the viscosities were 0.01400, 0.01412, 0.01408, and 0.01485cm$^2$/s, respectively. The lower viscosity shown here for 20% sucrose solution is slightly different from that which was
reported earlier (0.019 cm$^2$/s), because the ambient temperature in this experiment was higher (25°C) than reported values (20°C) from Josens and Farina (2001).

3.4 Discussion

When *S. invicta* is presented with a droplet of sucrose solution, the preferred method of feeding is suction, and liquid is stored in the crop. This behavior was expected, because fire ants are known to engage in trophallaxis between nest mates. Paul and Roces (2003) found that ants which share food with nest mates will choose suction as the preferred method of liquid feeding. Also, ants that use suction as their main liquid feeding method will often switch to the licking method when the droplet becomes a thin film on the surface (Paul and Roces 2003, Josens and Roces 2000). However, red imported fire ants were not observed to drink from a small amount of liquid at all. When our observations were made, a thin film was initially presented to hungry ants instead of watching the ant drink the entire droplet until the droplet became a film on the surface. Partial crop loads from unsubstantial food sources (dilute sucrose solutions) were recorded in the ant *C. mus* (Josens *et al.* 1998). *Solenopsis invicta* disregarding the small food source may be an indication of a similar behavior.

Initial weight of ants explained about 40% of the variability in total crop load for the constant concentration tests. Variables such as crop load and intake rate will vary with the size of ants within the same species (Paul and Roces 2003, Josens *et al.* 1998). The initial weights of individual ants were used to standardize crop loads (relative crop load). Total crop load was found to depend on viscosity as well as initial ant weight. As viscosity increased in a 30% sucrose solution, relative crop load decreased. As stated earlier, intake rate also decreased with increasing viscosity. Perhaps these ants left the
food source with partial crop loads, because the food source was not profitable enough to spend extensive time there, assuming that the ants fed at lower intake rates because of a mechanical obstacle due to high viscosities. This behavior was suggested and studied in honeybees (Schmid-Hempel et al. 1985, Núñez 1982) and leafcuffer ants (Roces and Núñez 1993). Unlike viscosity, however, sucrose concentration had no obvious effect on crop load in this study.

Cohen (2004) stated that viscosity is the most important textural characteristic in liquid diets. In *S. invicta*, solution intake rates, which ranged from 0.02971µl/min to 0.04748µl/min, decreased with increasing sucrose concentration in the BS. Ants imbibed dilute base solutions at the highest possible rate in this series, seemingly, because viscosity was not an obstacle. This is similar to results found by Josens and Farina (2001) with the hovering hawk moth, *M. stellatarum*, which would drink solutions at over 90µl/min. Fire ants reached a critical viscosity (0.25cm²/s) earlier than the hawk moth (0.35cm²/s) in the CCS probably because of the considerable difference size. *Solenopsis invicta* behavior does not mirror that, however, of the carpenter ant *C. mus* (intake rates can reach over 3µl/min) discussed earlier who imbibed dilute solutions at lower rates (Josens et al. 1998). When viscosity is held constant, intake rates are slow and constant. Given these findings, viscosity is a major factor in red imported fire ant feeding rates.

So far, our data has matched red imported fire ant feeding behavior closer to that of Lepidoptera and honeybees than that of *Camponotus*. More evidence pointing to this conclusion is found in sucrose intake rates. In *S. invicta*, these rates increase with increasing sucrose concentration. The maximum energy intake rate is found at the highest sucrose concentration solution tested (50%). Honeybees also maximize energy intake
close to this value (Harder 1986). Why do fire ants show feeding behavior more closely matching with honeybees than to *Camponotus*, a polymorphic ant species? *Camponotus mus* was starved for only two days in the study which found the ants fed slowly on dilute solutions (Josens et al. 1998). Josens and Roces (2000) found later that these rates on dilute solutions depend on colony starvation. *S. invicta* were starved for four days in this study. This may explain one difference in findings between the two ant species.

How much of the total crop load is explained by the size of an ant and viscosity of solution? In constant concentration tests, initial weight accounted for about 40% of total crop load whereas viscosity of solution accounts for varying amounts dependent on whether boric acid was present or not. Viscosity explained another 15% of total crop load in the CCS and 41% in the BCCS. When both variables were accounted for at the same time, a difference remained between the CCS in which only 46% variability was explained and the BCCS in which 54% of the total crop load was explained. The remaining variability may be attributed to individual motivation due to individual and colony dietary needs. According to these results, individual ants feeding from solutions without boric acid will leave with a total crop load dependent on 54% individual motivation. But when an ant feeds from a 0.5% boric acid solution, only 46% of the total crop load was dependent on individual motivation. Why were ants less selective at boric acid solutions? One explanation may be that boric acid solutions in this study acted as unique food sources. Before any bioassays were completed, colonies were allowed to acclimate to their new surroundings for about one month. These ants were fed 20% sugar water everyday, so when presented with sucrose solutions, ants were more likely to be
picky. This behavior has been well documented with *S. invicta* (O’Brien and Hooper-Bùi in press, Cassill and Tschinkel 1998).

Unlike CVS solutions 10-40%, the 50% sucrose solution (no Tylose) was imbibed at a significantly higher rate. This may indicate a small “Tylose effect” on behavior, even though ants were observed to readily consume sucrose solutions which contained Tylose. The ants may slightly detect the additive when present in higher concentrations, which negatively affects the feeding rate. The difference between intake rates of 10-40% sucrose solutions in the CVS and the rate at 50% sucrose solution is the measure of this Tylose effect. Furthermore, base solutions with boric acid (BA) were imbibed at higher rates than corresponding solutions in the BS. Does the addition of boric acid to sucrose solution make it easier for fire ants to drink (mechanically), or do the ants have an affinity for boric acid (chemosensorally)? If one assumes that the latter explanation is correct, then a more concentrated solution of boric acid in sucrose water might be even more attractive. However, Klotz *et al.* (1997) found that 5% boric acid in 10% sucrose solution showed significantly less consumption compared to 10% sugar water controls. Therefore, the mechanical explanation was more likely. From the viscosity measurements of boric acid solutions, we know the viscosity does not change with increases in boric acid concentration. Either another physical property such as surface tension is the reason for higher intake rates or this is another indication that ants preferred the unique food source.
CHAPTER 4: SUMMARY

How may red imported fire ant’s liquid feeding behavior affect the way ant baits are devised and prepared? Viscous baits on the market such as Terro Ant Killer may be initially attractive to *S. invicta*; however, other physical properties of liquid baits may decrease its effectiveness in the field. In Chapter 2, Terro may have revealed negative effects on feeding behavior after feeding had begun. In later experiments, ants were found to feed less and more slowly on viscous solutions, irrespective of sucrose concentration. However, ants consumed more toxicant overall when feeding on Terro than when feeding on the less viscous bait Dr. Moss. More toxicant in one foraging trip may be unfavorable if it decreases the chance of an ant performing trophallaxis upon return to the nest. Therefore, less viscous baits may be spread faster. If more attention is given to these watery liquid baits then a better delivery system will eventually be developed.

Viscosity was not the only property of liquid baits found to affect liquid feeding in these ants. Surprising behavior was observed with ants feeding from sucrose-boric acid solutions compared with behavior at sucrose solutions. At most sucrose concentrations, ants feeding from boric acid solutions drank faster than those ants feeding from solutions without the toxicant. It was hypothesized in Chapter 3 that boric acid either changes a physical property of sucrose solutions or represents a unique food source, first noted in Chapter 2. If ants feed faster just because the boric acid solutions were new, then the reverse situation is likely to be true. This may have major implications in the pest control industry. If a pest management professional is using a boric acid-based bait product (or other toxicant) to control fire ants, then the same ants may likely look for different food
sources over time if no change is made to treatments. Therefore, frequent changes to bait products used to treat ants should be made to maximize efficiency in eliminating this pest.
REFERENCES


APPENDIX: LETTER OF PERMISSION

October 6, 2005

Ms. Kathryn O'Brien
Red Imported Fire Ant Lab
LSU AgCenter
Baton Rouge, LA 70803

Dear Ms. O'Brien,

The Entomological Society of America grants you permission to reproduce the article cited below as part of your thesis for Louisiana State University.


Please provide proper acknowledgement.

Sincerely,

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Fax: 301-731-4538
akahan@entsoc.org

----- Original Message -----
From: Kathryn O'Brien
To: 'Alan Kahan'
Sent: Thursday, October 06, 2005 10:04 AM
Subject: RE: Publication as part of a thesis

Mr. Kahan:

Sorry, you must not have received my earlier request. May I use my manuscript that has recently been accepted in the Journal of Economic Entomology in my thesis? I do not have the citation as it has not been published yet. The manuscript is entitled "Hunger in Red Imported Fire Ants and Their Behavioral Response to Two Liquid Bait Products," (O'Brien and Hooper-Bùi in press). An e-mail will be fine. Thanks again.

Kathryn O'Brien
VITA

Kathryn Shea O’Brien was born on November 20, 1980, in Baton Rouge, Louisiana, and grew up in Prairieville, Louisiana, where she attended St. Amant High School. She received her Bachelor of Science in Biological Engineering at Louisiana State University in 2003 with a senior design project entitled “Design of an ant liquid bait station.” Kathryn then started her work toward a Master of Science degree under the supervision of Dr. Linda Hooper-Bùi. She published the first data chapter of her thesis entitled “Hunger in Red Imported Fire Ants and Their Behavioral Response to Two Liquid Bait Products” (In Press) in the Journal of Economic Entomology.