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COLONY GROWTH OF TWO SPECIES OF SOLENOPSIS FIRE ANTS (HYMENOPTERA: FORMICIDAE) REARED WITH CRICKETS AND BEEF LIVER

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ABSTRACT

Most diets for rearing fire ants and other ants contain insects such as crickets or mealworms. Unfortunately, insect diets are expensive, especially for large rearing operations, and are not always easily available or uniformly effective. This study was designed to examine colony growth of Solenopsis (Hymenoptera: Formicidae) fire ants fed beef liver. Five experiments were performed: four with Solenopsis invicta Buren colonies and one with Solenopsis geminata (F.) colonies. In these experiments, we compared the net growth of colonies fed raw liver, boiled liver-agar, and/or raw liver-agar all with house crickets (Acheta domesticus, (L.); Orthoptera: Gryllidae) as a standard diet. Both liver and house cricket diets produced healthy growing colonies at the end of 6-8 wk. However, colonies fed crickets were 1.7 to 3 times larger than those fed with liver. Raw liver and raw liver-agar diets performed similarly. Boiling the liver significantly reduced colony growth in S. geminata, but liver boiled for as long as 45 min still produced healthy fire ant colonies. This study demonstrates that beef liver is an acceptable diet for rearing laboratory colonies of both S. invicta and S. geminata for periods up to 6 months when maximum growth rates are not needed. However, house crickets are recommended for research studies where maximal growth is important because liver, mealworms, and banded crickets (Gryllodes sigillatus, F. Walker; Orthoptera: Gryllidae) are not as effective. Poor survival of liver-fed colonies after 8 months may indicate that beef liver is not an acceptable long-term diet. Pilot tests with canned liver cat food and a dry dog food showed they were palatable but not suitable for brood production in fire ant colonies.

Key Words: artificial diets, development, mass rearing, Solenopsis invicta, Solenopsis geminata, beef liver, Acheta domesticus, Gryllodes sigillatus

RESUMEN

La mayoría de las dietas para criar hormigas bravas y otras hormigas contienen insectos tales como grillos o gusanos de la harina. Desafortunadamente, las dietas de insectos son caras, especialmente al tratarse de operaciones de cría masiva y éstas no siempre están disponibles. Este estudio se diseñó para evaluar el crecimiento de las colonias de hormigas Solenopsis (Hymenoptera: Formicidae) alimentadas con hígado de res. Cinco ensayos fueron realizados: cuatro con colonias de Solenopsis invicta Buren y uno con colonias de Solenopsis geminata (F.). El crecimiento neto de las colonias alimentadas con hígado crudo, puré de hígado cocido en agar y / o puré de hígado crudo en agar, se comparó con el de las colonias alimentadas con grillos domésticos (Acheta domesticus, (L.); Orthoptera: Gryllidae) como un control estándar. Ambas dietas, las de hígado y grillo doméstico, produjeron colonias crecientes y sanas al final de 6-8 semanas. Sin embargo, las colonias alimentadas con grillos crecieron 1,7 a 3 veces más que las alimentadas con el hígado de res. Las dietas de hígado crudo y puré de hígado crudo en agar se comportaron de forma similar. El cocinar el hígado redujo significativamente el crecimiento en S. geminata, pero el hígado cocinado hasta por 45 min produjo colonias sanas de hormigas bravas. Este estudio demuestra que el hígado de res es una dieta aceptable para la cría de colonias de laboratorio, tanto de S.invicta como de S. geminata por períodos de seis meses, cuando las tasas máximas de crecimiento no son necesarias. Sin embargo, los grillos domésticos son recomendados para estudios de investigación donde el máximo crecimiento es importante, debido a que el hígado, los gusanos de la harina, y los grillos domésticos tropicales o listados asiáticos (Gryllodes sigillatus, F. Walker; Orthoptera: Gryllidae) no son tan efectivos. Además, la baja sobrevivencia de colonias alimentadas con hígado después de ocho meses puede indicar que el hígado de res no es una dieta aceptable a largo plazo. Las pruebas pilotos con comida de hígado enlatada para gatos y comida seca para perros demostraron que éstas eran apetecibles pero no eran adecuadas para la producción de cría en colonias de hormigas bravas.

Palabras Claves: dietas artificiales, desarrollo, cría masiva, Solenopsis invicta, Solenopsis geminata, hígado de res, Acheta domesticus, Gryllodes sigillatus
Several diets have been proposed for rearing imported *Solenopsis* fire ants (Khan et al. 1967; Bhattacharjee & Whitcomb 1970; Williams et al. 1980; Banks et al. 1981; Porter 1989; Vogt 2003). Most of these diets contain insects because fire ants are known to feed on insects and other small invertebrates (Vinson & Greenberg 1986). Fire ants also collect large quantities of sugary liquids (Tennent & Porter 1991), which are the main source of energy for adult workers due to their inability to ingest solid foods (Petralia & Vinson 1978; Williams et al. 1980; Glancey et al. 1981). A sugar food source significantly increases the size and growth rates of red imported fire ants (*Solenopsis invicta* Buren) in laboratory colonies (Williams et al. 1980; Porter 1989). In addition, studies of food flow in ant colonies have shown the importance of sugar solutions as fuel for workers, while protein is reserved for larval development (Brian 1973; Quinlan & Cherrett 1979; Howard & Tschinkel 1981; Sorensen & Vinson 1981; Brian 1983; Ali & Reagan 1985; Porter & Tschinkel 1985). Various studies have determined that insects are important for normal larval growth in fire ant colonies (Sorensen et al. 1983; Williams et al. 1987; Porter 1989). While the Bhattacharjee & Whitcomb (1970) agar diet with egg, honey, and a vitamin capsule has been widely used for rearing many species of ants (Buschinger & Pfeifer 1988; Hölldobler & Wilson 1990; Straka & Feldaar 2007), it is unsuitable for rearing fire ant colonies without insect supplements (Williams et al. 1980; Williams et al. 1987; Porter 1989; Vogt 2003; unpublished data).

Diets rich in insects like crickets can produce healthy young fire ant colonies, which grow in size by 1-2 orders of magnitude in only 2 months. With sufficient food, brood to worker ratios are usually well in excess of one (Porter 1988, 1989; Macom & Porter 1995). Unfortunately, such diets are expensive, especially for large rearing operations. Crickets and mealworms are time-consuming to rear and relatively costly to purchase. Furthermore, such insects are not always conveniently available when working in the field or travelling abroad.

This study was designed to examine the growth of fire ant colonies on beef liver after a pilot test showed promising results. Liver has been successfully used in diets for rearing insects in the orders Diptera, Hemiptera, Coleoptera and Neuroptera (Cohen 1985; Sherman & My-Tien Tran 1995; Arijs & De Clercq 2004). In addition, liver of other vertebrates such as rabbit, fowl, sheep, or ox has been used to rear the pharaoh's ant, *Monomorium pharaonis* (L.) (Peacock & Baxter 1949). Finally, beef liver was used in addition to crickets and mealworms by Tschinkel & Porter (1988) to maximize growth rate for testing the efficiency of sperm used by *S. invicta* queens, but no data were provided concerning the health or growth of test colonies. Here, we compared the growth of *Solenopsis* fire ant colonies fed beef liver that was either raw or pureed in agar. The overall objective was to find an inexpensive, convenient diet capable of rearing healthy growing fire ant colonies without the need for insect supplements. We were also interested in determining if cooking the liver affected colony growth by destroying essential diet nutrients (Seegers & Mattill 1935; Rice & Beuk 1953).

**Materials and Methods**

We used established colonies of both *S. invicta* and *S. geminata* reared from founding queens collected in Gainesville, Florida in June 2011. These colonies were fed only frozen adult house crickets (*A. domesticus*; Ghann Cricket Farm Inc., Augusta, Georgia) and sugar-water. Except where noted, test colonies were kept in 38 × 24 × 13 cm foraging trays (Del-Tech/Panel Controls 300-5N; Del-Tech Packaging), covered with clear plastic lids. Each foraging tray contained a 15 cm Petri dish nest and a 16 × 175 mm test tube nest. Water was continuously provided to each colony in a 16 × 150 mm test tube plugged with a cotton wad (Banks et al. 1981). Appropriate sanitary procedures were used to avoid contamination with *Solenopsis invicta* virus 3 (SINV-3) and other potential pathogens (Valles & Porter 2013). Test colonies were maintained at 28 ± 1 °C (SD), 34 ± 3% RH and approximately equal periods of light and dark.

The agar based diets were prepared once per month. They were poured into containers and allowed to set at room temperature for around 20 min, before being refrigerated. After 1-2 days of refrigeration, the solidified agar mixture was cut into small cubes of 1.5 ± 0.4 g (SD) and frozen at -20 °C to allow longer shelf life. All test diets were presented ad libitum in plastic weigh boats or 2 oz. plastic soufflé cups.

Sugar water was provided ad libitum to all test colonies in the form of «sugar wads,» unless otherwise specified. Small laboratory tissues (Kimwipes, Kimberly-Clark Worldwide Inc.) were wadded up, soaked in 2 M sucrose (684 g/L) and oven-dried at 50 °C. The sugar wads were kept on plastic lids or weigh boats and remoistened with tap water 3 days per wk (M-W-F). The sugar wads were added as required, or replaced if moldy. Sugar water was used because granular sugar, heavy syrups, and pure honey are generally ignored by fire ant foragers (Porter 1989).

**Diet Tests with *Solenopsis invicta* Colonies**

**Raw and Short-Boiled Liver Experiment.** We compared fire ant colony growth when fed 1) raw frozen liver, 2) pureed raw liver-agar, and 3) a
short-boiled, pureed liver-agar against 4) a standard house cricket diet. Thirteen small colonies (1 g brood, 0.5 g workers), and another 16 larger colonies (2 g brood, 1 g workers) were used, each with 1 mated queen. Test colonies were randomly assigned to treatments with an extra small colony in the cricket-standard diet treatment. Colonies were maintained from 19 Dec 2011 to 31 Jan 2012.

The cricket diet consisted of frozen adult house crickets. The raw frozen liver diet were maintained from 19 Dec 2011 to 31 Jan 2012. in the cricket-standard diet treatment. Colonies assigned to treatments with an extra small colony with 1 mated queen. Test colonies were randomly (1 g brood, 0.5 g workers), and another 16 larger standard house cricket diet. Thirteen small colonies short-boiled, pureed liver-agar against 4) a standard house cricket diet (A. domesticus) and the banded or tropical house cricket (G. sigillatus, Ghann’s Cricket Farm). This test was conducted for 7 wk (19 Jun to 5 Aug 2013) using methods similar to those in the first founding queen test above, except 1.5 M sugar water (43% sucrose by weight) was supplied continuously in 10 × 75 mm test tubes plugged by small sections of cotton wicks and rearing containers were 17 × 12 × 6 cm (R750B, Sterling King Products). Queens were collected in Gainesville, Florida on 20 May 2013.

Pilot Tests with Cat and Dog Food. A pilot test was conducted with canned cat food and another pilot test was conducted with dry dog food to see if these vitamin-enriched diets for vertebrate predators might also work with fire ants. Two kinds of canned cat food (Friskies Classic Paté Chicken-Liver and Friskies Classic Paté Turkey & Giblets, Purina) were each fed to 4 colonies as described in the long-boiled liver experiment above. We also fed dry dog food (Purina Dog Chow) to 20 small founding colonies as described in the first founding queen experiment above. The dog food was moistened 3 times per wk and was provided ad libitum.

Diet Tests with Solenopsis geminata Colonies

We compared the effectiveness of 2 liver-agar diets with another Solenopsis species the tropical fire ant, S. geminata. A pureed raw liver-agar diet and a short-boiled, pureed liver-agar diet were compared to a standard house cricket diet. The liver diets were prepared as described in the S. invicta raw and short-boiled liver experiment above. Fourteen colonies were used: 5 small colonies (1 g brood, 0.5 g workers); and 9 large colonies (2 g brood, 1 g workers), each with one mated queen. Three large and 2 small colonies were randomly assigned to each of the treatments except only 3 large colonies and one small colony were available for the boiled liver-agar treatment. Colonies were maintained from 4 Jan to 17 Feb 2012.

Other Species of Ants

We also explored the suitability of raw beef liver for rearing 5 other species of ants collected in North Florida in 4 genera and 2 subfamilies, Myrmicinae: Monomorium floricola (Jerdon), Monomorium pharaonis (L.), Pheidole dentata Mayr; and Formicinae: Nylanderia fulva (Mayr),
and *Camponotus floridanus* (Buckley). Two colonies were used for the *Monomorium* species and one for the others. These colonies were reared only on a liver and sugar water diet beginning 28 Dec 2012.

Variables Measured and Statistical Analyses

Net colony growth (final minus initial weights) and brood to worker ratio (final weight of brood divided by that of workers) were determined for each test colony unless otherwise specified. Workers and brood were separated using sorting sheets (Porter & Tschinkel 1985).

A two-factor General Linear Model Analysis of Variance (GLM 2-WAY ANOVA) was used to evaluate total (brood + workers) net colony growth and the brood to worker ratios for the *S. invicta* and *S. geminata* diet experiments. The factors were diet type and colony size. Total net growth was log-transformed to normalize the variance. Tukey-Kramer Multiple-Comparison Tests were performed to compare means. We used *t*-tests for unequal variance for the *S. invicta* long-boiled liver experiment and the founding queen experiments (Hintze 2001; NCSS 2001).

**RESULTS**

**Diet Tests with Solenopsis invicta Colonies**

**Raw and Short-Boiled Liver Experiment.** Workers readily approached and collected all of the diets presented. After 6 wk, the net growth of *S. invicta* workers and brood in the diet treatments was assessed (Fig. 1A). Four colonies were excluded from the analysis because they ceased to produce brood entirely by the end of the test period—something that never happens with healthy laboratory colonies. Three of these colonies were from the raw liver agar diet (2 large, 1 small) and one was from the raw liver diet (1 large). The loss of 3 colonies from the raw liver-agar diet was a bit puzzling, but it did not differ significantly from random ($\chi^2 = 6.4, df = 3, P = 0.0955$).

All remaining colonies were healthy and grew substantially (Fig. 1A). Diet treatments differed significantly in net growth ($F_{3,17} = 7.46; P = 0.0021$; GLM 2-WAY ANOVA), mainly because colonies that were fed house crickets were 70-200% larger than colonies raised with liver. Net growth of colonies fed raw liver was statistically intermediate to those fed house crickets and the 2 liver-agar diets (Fig. 1A; Tukey-Kramer tests). Colony size (Large versus Small) was also significant as a factor ($F_{1,17} = 4.65; P = 0.0456$); however, the interaction between colony size and diet was not significant. Separate analyses of only brood or only workers also revealed significant differences between colony sizes ($F_{1,16} = 4.71; P = 0.0455$ for brood; $F_{1,17} = 22.24; P = 0.0002$ for workers) and between diets ($F_{3,16} = 8.28; P = 0.0015$ for brood; $F_{1,17} = 3.60; P = 0.0352$ for workers). Brood to worker ratios (weight:weight) among these diet treatments and colony sizes were significantly different (see Fig. 1A; GLM 2-WAY ANOVA; $F_{3,17} = 4.92; P = 0.0122$; and $F_{1,17} = 4.91; P = 0.0406$; respectively). The average brood to workers ratios for the cricket, raw liver, short-boiled liver-agar, and raw liver-agar diets were accordingly 3.25, 2.16, 1.74, and 1.77 (large and small colonies combined). Brood to worker ratios for the large and small colonies (combined across treatments) were respectively: 1.83 and 2.64.

**Long-Boiled Liver Experiment.** Colony net growth rates were similar to the raw and short-boiled liver experiment (Figs. 1A and 1B). Net growth of colonies fed house crickets was about twice that of colonies fed the long-boiled liver-agar diet (52 and 24 g, respectively); but the difference was not significant because of high colony variability (3.7, 12.7, 42.0, and 49.3 g for long-boiled liver-agar compared to 49.8, 55.8, and 59.8 g for those colonies fed house crickets; Fig. 1B; $t = 1.83, P = 0.16$).
Founding Queen Experiments. In the first founding-queen experiment, the total net growth of newly founded *S. invicta* colonies (brood + workers) was 1.5 times larger when fed house crickets rather than liver after 8 wk ($1.13 \pm 0.05$ vs. $0.77 \pm 0.05$ g $\pm$ SE; $t = 4.75$, $P = 0.00004$; $n = 20$ and 19). One of the 20 liver colonies was eliminated because the queen died early in the experiment.

In the second experiment, the total net growth of newly founded *S. invicta* colonies after 7 wk was 4.5 times larger when fed house crickets (*A. domesticus*) rather than banded crickets (*G. sigillatus*) ($1.12 \pm 0.15$ vs. $0.25 \pm 0.07$ g $\pm$ SE; $t = 5.17$, $P = 0.00005$; $n = 15$ and 16). Five colonies were eliminated from the treatments (3 and 2, respectively) because the queen died, sexual brood was produced, or brood production ceased entirely.

Pilot Tests with Cat and Dog Food. Workers readily collected both the canned cat food and the dry dog food but neither diet produced healthy fire ant colonies after 6-10 wk. Colonies fed canned cat food (both varieties) grew 20-45% after 6 wk compared to more than 10-fold for colonies fed house crickets (GLM 1-WAY ANOVA; $F_{2,11} = 261$; $P < 0.0001$). Furthermore, brood to worker ratios and the percentage of pupae in the brood of the cat food colonies were less than half those found in the cricket colonies. Founding colonies fed dog food weighed only 5% of the weight of colonies fed house crickets after 10 wk ($0.058 \pm 0.006$ vs. $1.13 \pm 0.05$ g $\pm$ SE; $t = 21.0$, $P < 0.0001$; $n = 19$ and 20).

Diet Tests with *S. geminata* Colonies

Results for the raw and boiled liver diet test with *S. geminata* were similar to those with *S. invicta* in that colonies fed house crickets grew faster and were 70-200% larger than colonies fed raw liver-agar or boiled-liver agar (Fig. 2; $F_{2,8} = 40.05$; $P < 0.0001$; GLM 2-WAY ANOVA). Colonies that received cricket and liver diets were healthy and grew substantially as was seen with *S. invicta* (Fig. 1), but each of the diet treatments fed to *S. geminata* differed significantly in net growth (Fig. 2; Tukey-Kramer test). As expected, total net growth for the larger colonies was significantly greater than for the smaller colonies ($F_{1,6} = 47.14$; $P = 0.0001$), but there was no interaction between colony size and diet. Brood to worker ratios (weight:weight) between these diets were statistically different (2.61, 1.63, and 1.41 for crickets, raw liver-agar, and boiled liver-agar diets respectively; $F_{2,8} = 7.47$; $P = 0.0148$; Tukey-Kramer test).

Brood to worker ratios for large and small colonies ($1.82 \pm 0.16$ vs. $1.95 \pm 0.21$) did not differ significantly, nor was there an interaction between these 2 factors (diet vs. colony size).

Long-Term Suitability of a Liver Diet

Ten of the 19 *S. invicta* colonies, fed raw frozen liver from the founding queen experiment, were retained for long-term monitoring. Sixty percent of these colonies (6/10) were still healthy and growing after 5.5 months compared to 80% of colonies (16/20) receiving crickets. However, after 9 months only 20% (2/10) of the liver colonies were still producing brood compared to 75% (15/20) of the cricket colonies ($\chi^2 = 8.2$, $df = 1$, $P = 0.004$). A pilot test with eight established colonies raised on pure raw liver-agar the year before produced similar results in that 75% (6/8) of the colonies were healthy for the first 5 months but all had stopped producing brood after 9 months.

Other Species of Ants

Three species of ants (*M. pharaonis*, *M. floridula*, *C. floridanus*) were still healthy and growing with normal amounts of brood after almost 8 months on a diet of only raw frozen beef liver and sugar water (Aug 2013). The *P. dentata* colony and the *N. fulva* colony were healthy for 3 and 4 months, respectively, before brood production ceased for unknown reasons.

DISCUSSION

A diet of beef liver consistently produced healthy growing fire ant colonies (both *S. invicta* and *S. geminata*) with brood to worker ratios $> 1.5$. Colonies fed house crickets (*A. domesticus*) grew 70 to 200% larger than those receiving liver (Figs. 1 and 2); nevertheless, liver may be as good as some insect diets because incipient *S. invicta* colonies fed liver in the first founding queen experiment had a growth trajectory as good as or better than incipient colonies fed *Zophobas* mealworms (*Tenebroididae*) in a parallel study of the
importance of salt in fire ant diets (Resasco 2013). Similarly, colonies fed liver in the first founding queen experiment grew at least twice as fast as colonies fed banded crickets (G. sigillatus) in the second founding queen experiment. While house crickets (A. domesticus) are a superior diet when compared to liver, house crickets are also about eight times more expensive than beef liver ($64 vs. $8/kg) and not always readily available. Beef liver, raw and pureed in agar are both suitable for rearing fire ants, but raw liver is probably best because it is ready to be used and performed as well or better than the agar presentations. Canned cat food containing liver and meat by-products and a common brand of dry dog food, however, were not suitable diets for fire ants, perhaps due to nutrient and/or preservative content.

While liver diets maintained healthy growing colonies for periods of 4-6 months, more tests are required before recommending beef liver as a sole diet for rearing fire ant colonies for longer periods. Almost all colonies from both the founding queen test and a pilot test showed poor brood production after eight months when compared to colonies fed house crickets. Possibilities for poor brood production include a micronutrient imbalance, a build up of toxins from the liver, or effects of an unknown pathogen. Whatever the cause, the effects seem most likely to be on the queen because colonies had grown considerably in size over a period that included more than 6 brood cycles and at least 2 complete replacement cycles of workers (egg to worker death) (Porter 1988; Calabi & Porter 1989).

Fire ant colonies fed raw or fried ground beef produced un-pigmented workers after 66 d in a study conducted by Williams et al. (1987), but colonies in our study never showed this pathology when fed beef liver. Porter (1989) reported that an artificial diet including boiled eggs, vegetable puree and a modified Bhatkar diet with cooked ground beef produced no colony growth. Porter (1989) speculated that cooking may have destroyed essential nutrients. In this study, we found that boiling the beef liver for short periods of time seemed to reduce its nutritive value (Figs. 1 and 2), but boiling the liver even for a long period still produced healthy growing colonies (Figs. 1 and 2). In other words, the temperature and perhaps the duration of boiling may diminish, but apparently do not destroy nutrients in beef liver (Seegers & Mattill 1935; Rice & Beuk 1953), which are essential for the growth of healthy fire ant colonies.

In conclusion, house crickets (A. domesticus) outperformed all liver diets. Nevertheless, beef liver is a good substitute or supplement for house crickets for rearing healthy fire ants if maximal growth rates are not essential (Figs. 1 and 2). Raw liver was as good as or better than Zophobas mealworms or banded crickets. Nevertheless, use of only beef liver and sugar water to rear fire ants for more than 6 months is not advised pending results of additional long-term diet tests. We were also able to successfully rear 5 other species of ants in 4 other genera for at least 3-8 months with raw beef liver indicating that liver may be useful for rearing ants in other genera as well.

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