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Authors: Porter, Sanford D., Valles, Steven M., and Gavilanez-Slone, Jenny M.

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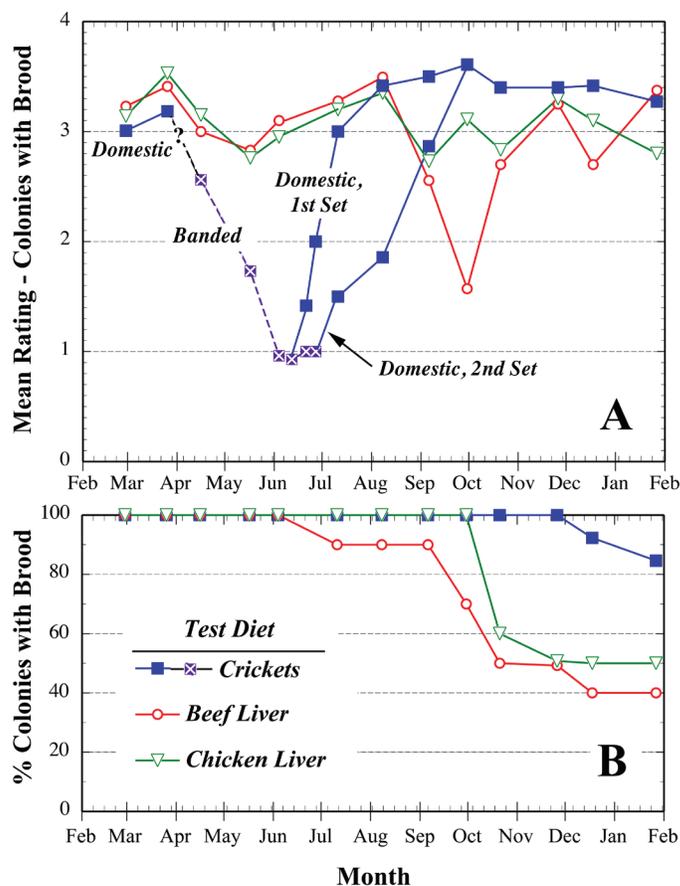
# Long-term efficacy of two cricket and two liver diets for rearing laboratory fire ant colonies (Hymenoptera: Formicidae: *Solenopsis invicta*)

Sanford D. Porter\*, Steven M. Valles, and Jenny M. Gavilanez-Slone

Effective diets are necessary for many kinds of laboratory studies of ants (Porter 1988; Macom & Porter 1995; Feldhaar et al. 2007; Valles et al. 2013). Unfortunately, artificial diets including Bhatkar's diet (Bhatkar & Whitcomb 1970; Hölldobler & Wilson 1990) have proven ineffective at rearing healthy fire ant colonies (Vogt 2003; Gavilanez-Slone & Porter 2014). Gavilanez-Slone & Porter (2013) recently showed that raw beef liver and sugar water is a suitable diet for rearing healthy growing fire ant colonies for 4 to 6 mo if maximum rates of growth are not needed. They reported that 2 species of fire ants (*Solenopsis invicta* Buren and *Solenopsis geminata* [F.]; Hymenoptera: Formicidae) grew several times faster when fed domestic crickets (*Acheta domesticus* [L.]; Orthoptera: Gryllidae) rather than beef liver. However, beef liver is only about 1/8 the price of crickets and is much more readily available throughout most of the world than are domestic crickets.

The primary objective of this note is to report the long-term efficacy of using a liver and sugar water diet to rear fire ants. This note also provides information about the relative usefulness of raw chicken liver and banded crickets (*Gryllobates sigillatus* [Walker, F.]; Orthoptera: Gryllidae) for rearing fire ants.

The test was started on 25 Jan 2013 and terminated 1 yr later. We used *S. invicta* colonies that were about 7 mo old and had been reared on domestic crickets and 1.5 M sugar water. These test colonies initially contained a queen, 2 to 3 g of workers (3,000 to 4,000), and 2 to 3 g of brood. Test colonies were placed in small 17 × 12 × 6 cm nest boxes (R750B, Sterling King Products, Lyons, Illinois, USA). Ants were contained in the boxes by painting the sides with a Fluon-like suspension of fluoropolymer resin (Daikin Polyflon PTFE DX-9025; Daikin America, Inc., Orangeburg, New York, USA) and by snapping on a tight-fitting lid dusted with talcum powder. The lid had a 6 × 12 cm rectangle cut out to allow access for feeding and colony ratings (see Fig. 1 for rating scale). Each box initially contained two 125 × 16 mm test tube nests, but additional nest tubes were added as the colonies grew. Colonies were maintained at 28 °C, the middle of the growth window for this species (25–32 °C; Porter 1988) and manually reduced to 4 to 6 g every 2 to 3 mo so they did not outgrow their nest boxes. Appropriate sanitary procedures were taken to avoid contamination with the SINV-3 virus, including strict measures to avoid mechanical vectoring between test colonies (Valles & Porter 2013). To check for SINV-1, -2, and -3 virus infections (Valles et al. 2009), samples of 15 live workers were taken from each colony shortly after brood production ceased or, if the colony remained healthy, at the end of the study. These samples were stored at -70 °C until analysis.



**Fig. 1.** Results of a 1 yr diet study using sugar water and either raw beef liver, raw chicken liver, or crickets to rear colonies of the imported fire ant *Solenopsis invicta*. A) Mean brood production rating of test colonies with brood: 4 = excellent, substantially more brood than workers; 3 = good, brood about equal to workers; 2 = poor, brood substantially less than workers; 1 = bad, only a little brood visible; and 0 = no brood. Colonies were fractionally rated if they appeared intermediate. Early in the study, about the end of Mar, we switched from domestic crickets (black squares) to banded crickets (crossed squares) and then back to domestic crickets (black squares) for 6 ant colonies on 6 Jun and then the remaining 7 colonies on 1 Jul. Colonies were eliminated from rating means when the queen died or brood production ceased. B) Percentage of test colonies containing brood plotted against time in months for colonies receiving either beef liver ( $N=10$ ), chicken liver ( $N=10$ ), or crickets ( $N=13$ ).

Center for Medical, Agricultural and Veterinary Entomology, USDA-ARS, 1600 SW 23rd Drive, Gainesville, FL 32608, USA

\*Corresponding author; E-mail: sanford.porter@ars.usda.gov

Colonies were fed 3 times a week: 10 colonies each received 0.45 ± 0.10 g (± SD) frozen raw chicken liver, 10 colonies received the same amount of frozen raw beef liver, and 13 colonies each received 2 frozen crickets (0.65 ± 0.15 g). Domestic crickets (*A. domesticus*) were used for the first month or two of the test, but we switched to banded crickets (*G. sigillatus*) when our supplier switched species because of rearing problems associated with *Acheta domesticus densovirus* (AdDNV; Szelei et al. 2011). AdDNV appears to be specific to *A. domesticus* (Szelei et al. 2011) because Lepidoptera were not infected and 2 species of crickets, including the banded cricket (*G. sigillatus*), were resistant (Szelei et al. 2011). This virus does not appear to replicate in fire ants (*S. M. Valles*, unpublished data). All colonies received sugar water in a 10 × 75 mm tube plugged with an approx. 1 cm long piece of 5/16 inch cotton wick (Richmond Dental, Charlotte, North Carolina, USA). New sugar tubes were added about once per week. We used 1.5 M sugar water (approx. 43% sucrose by weight) because this concentration was high enough to resist mold and fermentation for several weeks, but low enough that the sugar usually did not crystallize on the cotton before being consumed.

The results showed that banded crickets were not suitable for long-term rearing of fire ants (Fig. 1A). By late May, all cricket-fed colonies in this study were in very poor or bad condition, having minimal amounts of brood (Fig. 1A). In contrast, all of the liver colonies contained healthy amounts of brood. However, after 6 of the cricket-fed colonies were switched from banded crickets back to domestic crickets (on 6 Jun), brood production improved dramatically over a period of about 1 mo. This improvement was significantly different from control colonies still receiving banded crickets (Fig. 1A; 12 Jun versus 27 Jun;  $N = 6$  and  $7$ , respectively; Mann Whitney U test on rating change,  $Z = 2.99$ ,  $P = 0.0034$ ). Brood production improved similarly (Fig. 1A) when the 2nd set of the cricket-fed colonies ( $N = 7$ ) were switched back to domestic crickets (on 1 Jul), although the recovery period was about 2 mo. A subsequent test, initiated by the results seen in Fig. 1, showed that incipient fire ant colonies reared from founding queens grew very poorly when fed banded rather than domestic crickets (Gavilanez-Slone & Porter 2013).

For about 8 mo, colonies fed crickets, raw beef liver, and raw chicken liver all had similar proportions of colonies producing brood (Fig. 1B). However, by 10 mo (late Nov), a substantial difference in survival existed among treatments (Fig. 1B): half of the colonies receiving either the beef liver diet (5/10) or the chicken liver diet (5/10) had stopped producing brood compared with 0% of colonies (13/13) fed crickets (Fisher exact  $2 \times 3$  probability test of the proposition that colonies producing and not producing brood were randomly distributed among treatments,  $P = 0.0044$ , 2-tailed test). At the end of 12 mo (Feb), however, the distribution of healthy colonies among the treatments was still strongly skewed to the cricket colonies (Fig. 1B) but did not quite differ from random because 2 of the cricket colonies had also stopped producing brood (Fisher exact  $2 \times 3$  probability test as above,  $P = 0.094$ ). Nevertheless, when the beef liver and chicken liver treatments were combined for a 1-tailed test of the proposition that more colonies stopped producing brood when fed liver than when fed crickets, the results were still significant ( $2 \times 2$  Fisher exact test,  $P = 0.026$ ).

Well fed fire ant laboratory colonies do not normally stop producing brood as long as temperatures remain at or above 25 °C and below 32 °C (Porter 1988), unless the colonies are diseased or seriously stressed in some way. Once brood production stops in laboratory colonies, it rarely or never resumes. None of the colonies tested positive for SINV-1, -2, or -3, so the brood loss observed in this study (Fig. 1B) cannot be attributed to these viruses. A previous study with beef liver also reported declines in the proportions of colonies producing brood between 5 and 9 mo (Gavilanez-Slone & Porter 2013).

The causes of brood production failure associated with long-term use of liver diets in this study (Fig. 1) and the previous study (Gavilanez-Slone & Porter 2013) are unknown but could be related to missing micronutrients, unknown pathogens, the accumulation of toxins, or a combination of these and other unknown factors. If nutrient or toxin stresses do occur with liver diets, these stresses would need to accumulate primarily in the queen because the workers turn over 3 to 4 times a year at 28 °C (Calabi & Porter 1989). Colony variability is also important because, after the die-off in Oct and Nov, the remaining beef and chicken liver colonies were healthy through the last 2 mo of the study (Fig. 1). Also, fire ant colonies in our lab have been maintained successfully on liver for as long as 21 mo. Clearly, mechanisms causing brood loss in fire ant colonies on long-term liver diets will need to await future research.

## Summary

Fire ant colonies thrived on diets of sugar water and either raw beef liver or raw chicken liver for almost 8 mo, after which brood production ceased in half of these colonies. As expected, colonies did well on a diet of sugar water and domestic crickets. Unexpectedly, however, banded crickets were much less suitable than either liver or domestic crickets.

**Key Words:** *Acheta domesticus*; *Grylloides sigillatus*; banded cricket; brood production; raw liver; red imported fire ant

## Sumario

Colonias de hormigas bravas prosperaron en las dietas de agua azucarada y de hígado de pollo crudo o hígado de res crudo por casi ocho meses, tras lo cual la producción de cría se redujo a la mitad en las colonias criadas con hígado. Como era de esperarse, las colonias crecieron muy bien en una dieta de agua azucarada y grillos domésticos. Inesperadamente, sin embargo, los grillos domésticos tropicales o listados asiáticos fueron mucho peores que el hígado o grillos domésticos.

**Palabras Clave:** *Acheta domesticus*; *Grylloides sigillatus*; producción de cría; hígado crudo; hormiga brava

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