ATTRACTIVENESS AND EFFECTIVENESS OF AN ARTIFICIAL DIET FED TO HYBRID IMPORTED FIRE ANTS, *SOLENOPSIS INVICTA* × *RICHTERI* (HYMENOPTERA: FORMICIDAE)

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ABSTRACT

Attractiveness of freeze-dried and reconstituted entomophage diet to hybrid fire ants (*Solenopsis invicta* × *richteri*) was investigated in choice tests using freeze-killed, crushed cricket (*Acheta domestica* L.) as a standard. Worker ants were strongly attracted to both crickets and reconstituted diet. Foragers collected approx. 27 times more reconstituted diet than freeze-dried diet, and collected statistically equivalent amounts of artificial diet and crickets (36.0 ± 7.0 and 26.0 ± 0.3 mg/h, respectively). Even though workers were strongly attracted to the artificial diet, all measures of colony growth (mean mass of brood, workers, and queen) were at least 30% lower in colonies fed sugar water + artificial diet than in colonies fed sugar water + crickets or sugar water + artificial diet + crickets. While this diet may have some utility as a bait for monitoring fire ants in the field, it offers no advantage over a standard diet of crickets and sugar water for rearing fire ants in the laboratory.

Key Words: Colony growth, laboratory rearing, foraging, bait

RESUMEN

La atracción de alimentos entomófagos reconstituidos y liofilizados hacia *Solenopsis invicta* × *richteri* fue investigada en un experimento de preferencia usando como un grillos (*Acheta domestica* L.) molidos y matados por congelación. Las hormigas trabajadoras fueron fuertemente atraidas de igual manera hacia los grillos y el alimento reconstituido. Las hormigas colectoras de alimentos colectaron la dieta reconstituida aproximadamente 27 veces más que la dieta liofilizada, y colectaron estatisticamente una misma suma de dieta artificial y grillos (36.0 ± 7.0 y 26.0 ± 0.3 mg/h, respectivamente). Aunque las hormigas trabajadoras són fuertemente atraidas a la dieta artificial, todas las medidas del crecimiento de la colonia (promedio de cantidad de cría, trabajadoras, y reina) fueron por lo menos 50% más bajas en las colonias alimentadas con agua zucarada+dieta artificial en comparación con colonias alimentadas con agua azucarada+grillos o agua zucarada+dieta artificial+grillos. Mientras que esta dieta puede tener algun utilisaje como cebo para el chequeo de hormigas de fuego en el campo, no ofrece ninguna ventaja sobre la dieta de grillos y agua azucarada para la crianza de hormigas en el laboratorio.

Translation provided by Demian Kondo.

Various diets have been proposed for rearing imported fire ants (*Solenopsis invicta* Buren, *Solenopsis richteri* Forel, and *Solenopsis invicta* × *richteri*, the red, black, and hybrid imported fire ants, respectively) (Khan et al. 1967; Bhatkar & Whitcomb 1970; Banks et al. 1981; Porter 1989); however, none have proven satisfactory without whole insects, offered separately or as a diet component.

I tested the attractiveness of a liver and ground beef-based artificial entomophage diet (Cohen, U.S. Patent #5,834,177. November 10, 1998) to foraging hybrid imported fire ants, *S. invicta* × *richteri*. For a description of the diet, see Cohen & Smith (1998). This diet was tested because it has been used to successfully rear several generations of *Chrysoperla rufilabris* (Burmeister). A reasonable start to assessing potential benefit of the diet to fire ant colonies would be to test its palatability; thus, we addressed the following questions: Does attractiveness of the diet warrant further study on its use as a supplement for laboratory colonies? Is the diet more attractive in its freeze-dried form or its reconstituted form? Is fresh diet more attractive than freeze-dried and reconstituted diet? Finally, an experiment was conducted to determine growth of laboratory colonies fed the artificial diet, crickets (*Acheta domestica* L.), or a combination of both.

MATERIALS AND METHODS

Diet Attractiveness

Colonies of *S. invicta* × *richteri* were collected from the field (Oktibbeha Co., MS) and maintained in trays (56 cm L × 44 cm W × 12 cm H) with castone nests (150 mm × 25 mm), a water source (150 mm × 25 mm test tube filled with

water, and plugged with cotton), and 1 M sucrose solution in a 150 mm × 15 mm test tube plugged with cotton. All colonies had a functional queen, 50,000-100,000 workers, and 15-20 g of brood. Colonies were fed crickets 2×/week, occasionally supplemented with boiled hen's egg yolk. Crickets and egg yolk were removed from the colonies 2 d prior to all tests to insure uniform levels of hunger. All trials took place in a climate-controlled room (28°C, approx. 60% RH). Hybrid status of colonies was confirmed by chemotaxonomy (Vander Meer & Lofgren 1990).

In experiment 1, freeze-killed crickets, freeze-dried entomophage diet, and reconstituted entomophage diet (2:1 diet:water by weight) were tested for recruitment time and attractiveness to foraging ants. Crickets were macerated using a mortar and pestle prior to testing. Macerated crickets and reconstituted diet were similar in consistency, with the exception of some small (<3 mm^2) pieces of exoskeleton in the macerated crickets; freeze-dried diet was composed of fine (5 to 40 µm) particles with relatively few larger, stringy solids. Four test colonies were used; each was connected to a foraging arena (41.75 cm L × 27.5 cm W × 12 cm H tray) with Tygon® tubing. Diets were placed in the barrels of 10 ml syringes cut at the 7 cc mark to present a 154 mm^2 surface area of diet. Each syringe contained 3 cc of the appropriate diet. Syringes were placed in the foraging arena, equidistant to the arena entrance (approx. 15 cm). A pair of observers, each observing 2 colonies, recorded discovery time and recruitment time for each syringe. Recruitment was assumed to have taken place once 10 foragers were present at the food surface. Once recruitment had taken place, the number of ants on the surface of the food sources was estimated at timed intervals. As foragers removed material from the syringes, the plunger was pushed forward so that the surface area presented to foragers remained constant.

In experiment 2, freeze-dried and reconstituted entomophage diets were presented in 1 oz plastic soufflé cups. Each cup had 2 small (approx. 3 mm dia.) holes cut in the side for ant access, and a plastic lid to minimize desiccation of the material. Cups containing diet were preweighed to the nearest 0.01 g, dried for 24 h at 60°C and re-weighed. Water (Millipore) was then added to the reconstituted diet treatment (2:1 water:diet ratio), exposed to the ants, weighed again, then dried for 24 h at 60°C to obtain dry weight of material removed. Eight controls (4 freeze-dried and 4 reconstituted) were placed in the room outside of the ant colonies. Paired cups were placed directly in colony trays; care was taken to place the cups equidistant from nest cells. Ants were allowed to forage for approx. 5 h (exact time noted for each cup), then all cups with were removed along with their contents, dried, and weighed.

In experiment 3, foraging ants were allowed access to reconstituted entomophage diet and a cricket standard, to compare attractiveness/retrieval rate. Prior to conducting this experiment, samples of macerated cricket (N = 6) were weighed, dried for >24 h at 60°C, and reweighed to obtain water content. Data were used to express retrieval rates in terms of dry weight. Presentation of food sources was done in the same manner as in experiment 2.

In experiment 4, freeze-dried, reconstituted diet (commercially prepared and canned approx. 3 yr. prior to testing) and fresh diet (made the day prior to testing) were presented to foraging ants using the same methods as in experiment 2. Water content of fresh and reconstituted material was obtained by weighing samples of each (N = 3), drying them in a 60°C oven for >24 h, and reweighing them.

Discovery and recruitment data were subjected to Proc Mixed (Little et al. 1996) to test for differences between treatments, with colony as a blocking factor. Timed observations from experiment 1 were analyzed as a randomized complete block with repeated measures and subjected to Proc Mixed to test for differences in attraction between treatments, and changes in attraction over time. Data from the other experiments were analyzed using Proc Mixed with source colony as a random blocking factor to test for differences in retrieval or attractiveness between treatments. Analysis of variance was used to examine controls for differences between treatments. Data are presented as mean ± SE, and were tested for significance at the $\alpha = 0.05$ confidence level.

Growth of Laboratory Colonies

An experiment was designed to compare colony growth of hybrid fire ant colonies fed sugar water (SW) + crickets (C), SW + artificial diet (AD), and SW + C + AD. Colonies (n = 15) were collected from the field (Oktibbeha Co., MS) and standardized just prior to beginning the experiment. Each standard colony contained 1 physogastric queen, 5 g workers, and 2 g brood. Colonies were housed in trays (41.75 cm L × 27.5 cm W × 12 cm H) and provided a castone® nest (150 mm × 25 mm), water, and 1 M sugar solution. Crickets and artificial diet were offered separately in 1 oz. plastic soufflé cups, with small (0.3 mm) holes drilled in the side for forager access, and lids to slow desiccation of the contents. Cups were checked daily for mold or desiccation, and replaced as necessary. Colonies were provided the appropriate foods *ad libitum* for a total of 8 wk, replacing diet cups at least every 2 d. The experimental design was a completely randomized design replicated 5 times. Data were analyzed using Proc Mixed followed by Least Squares Means to test for treatment effects on queen mass, total

brood mass, total worker mass, and total colony mass (live weights). Data are reported as mean ± SE.

RESULTS

Diet Attractiveness

Experiment 1. Foraging ants discovered all food sources in <4 min, and recruited within <13 min. No significant differences existed in discovery or recruitment times ($P > 0.05$), which were quite variable (e.g., recruitment to freeze-dried diet ranged from 5.9 to 12.8 min). In a mixed model with colony, treatment (food type), and time as fixed effects, and colony by treatment as the subject of the repeated statement, treatment ($F = 36.2$; df = 2,6; $P = 0.0004$) and time (h) ($F = 4.9$; df = 22,198; $P < 0.0001$) significantly influenced number of foragers per bait (Fig. 1).

Experiment 2. Pre- and post-drying weights of freeze-dried diet indicated 2 to 6% water content, so dry weight after exposure to the ants was subtracted from dry weight prior to exposure to obtain amount of diet retrieved. Data were corrected for time exposed to foragers, yielding material retrieved in g/h. Foragers removed approx. 27 times more reconstituted diet from the cups than freeze-dried diet in terms of dry weight ($F = 28.5$; df = 1,7; $P = 0.001$) (Fig. 2). Controls (freeze-dried and reconstituted) remained unchanged during the course of the experiment.

Experiment 3. Freeze-killed crickets used in this trial averaged 67.1 ± 0.4% water. Based on time-corrected data, entomophage diet controls gained an average of 2.4 ± 1.0 g/h, and cricket controls lost an average of 2.4 ± 0.4 g/h; these amounts were applied to post-feeding dry weights as a correction factor. Dry weight of material retrieved by the ants was statistically indistinguishable for the two treatments ($F = 4.45$; df = 1,7; $P = 0.073$) (Fig. 3).

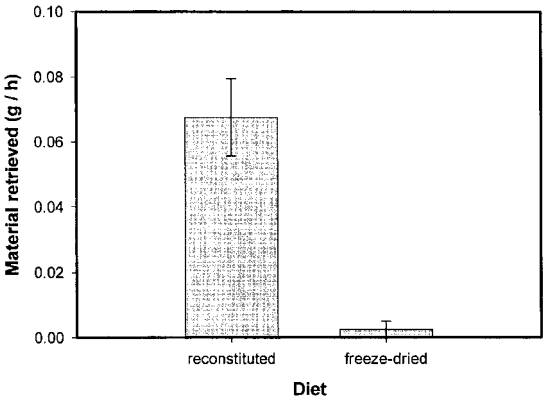

Fig. 2. Retrieval rate (g dry weight/h) for *S. invicta* × *richteri* foraging on reconstituted v. freeze-dried entomophage diet.

Experiment 4. Fresh entomophage diet contained an average of 68.7 ± 0.9% water, while reconstituted diet contained a statistically indistinguishable average of 71.7 ± 0.3% water ($P = 0.12$). Controls lost an average of 0.03 g during the course of the trial; this amount was the same between treatments ($P = 0.10$), and was subtracted from post-feeding data. Since water content of the treatments was similar, I analyzed wet weight of diet retrieved. In approx. 4 h, foragers collected similar amounts of fresh and reconstituted diet (0.49 ± 0.11 g and 0.40 ± 0.08 g, respectively) ($P = 0.09$).

Growth of Laboratory Colonies

Eight weeks after beginning the experiment, all measures of colony fitness and/or growth were significantly lower in colonies fed SW + AD than colonies fed SW + C or SW + AD + C (Table 1). Growth of colonies fed SW + AD appeared to keep

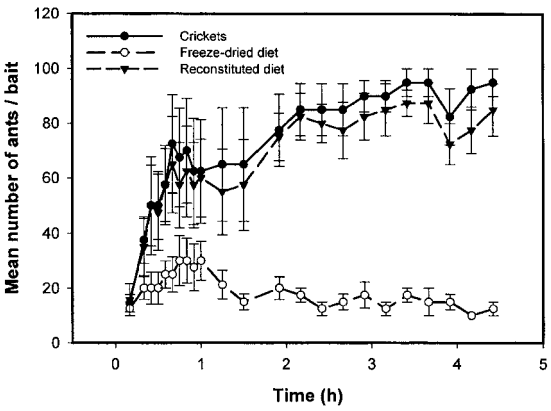

Fig. 1. Foraging activity of *S. invicta* × *richteri* during timed observations at 3 food sources.

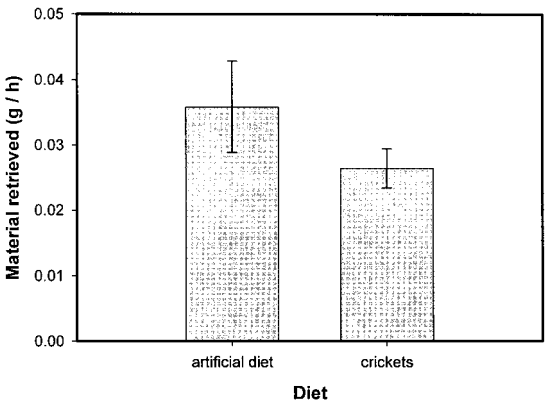

Fig. 3. Retrieval rates (g dry weight/h) for *S. invicta* × *richteri* foraging on entomophage diet v. crickets.

TABLE 1. MEAN (±SE) MASS OF HYBRID IMPORTED FIRE ANT COLONIES FED 3 DIFFERENT DIETS FOR 8 WK.

Treatment	Queen mass (mg)	Brood mass (mg)	Worker mass (mg)	Total mass (mg)
Sugar water + crickets	20.0 ± 2.0 a[1]	24.0 ± 5.1 a	15.1 ± 3.0 a	39.1 ± 7.8 a
Sugar water + artificial diet	14.1 ± 1.3 b	4.3 ± 4.3 b	5.6 ± 3.7 b	9.9 ± 7.9 b
Sugar water + crickets + artificial diet	24.1 ± 1.3 a	23.2 ± 3.9 a	10.6 ± 2.2 ab	33.7 ± 5.8 a

[1]Means in a column followed by the same letter are not significantly different (Least Squares Means, $P > 0.05$).

pace with growth in other treatments until approx. 4 wk into the experiment, but data were only collected at 8 wk. No apparent differences in worker size, color, or behavior were noted at the end of the experiment.

DISCUSSION

Discovery, recruitment, and retrieval rates indicate that the entomophage artificial diet is readily taken by laboratory fire ant colonies. Actual consumption was not measured, but foragers appeared to store large amounts of the diet in and around nest cells. Reconstituting the diet prior to presenting it to colonies increased the rate of retrieval. Low P-value for the analysis in experiment 4 suggests that fresh diet may be slightly more attractive than reconstituted diet; however, foragers were highly attracted to reconstituted diet in all experiments.

Fire ant colonies denied insect prey may cannibalize larvae (Sorensen et al. 1983) or produce abnormal, unmelanized workers (Williams et al. 1987). Workers appeared normal at the end of the colony growth study (e.g., no apparent change in color or size). While workers were not observed cannibalizing larvae during the experiment, that behavior could have contributed to the sharp decline in brood for colonies fed SW + AD. The decline in brood could explain lower queen weight in those colonies, as queen fecundity and ovarian development is tightly linked to presence of 4th instar larvae (Tschinkel 1995). The total mass of colonies fed SW + AD was 75% lower than mass of colonies fed SW + C. Porter (1989) reported that colonies fed crickets, sugar water, and an artificial diet based on Bhatkar and Whitcomb (1970) also exhibited growth indistinguishable from colonies fed crickets and sugar water only.

The artificial entomophage diet tested in these studies may have some utility as an attractive bait for monitoring fire ant presence or activity in the field, or as a hydratable carrier for toxins in home bait stations; however, it offers no advantage alone or in combination with a standard cricket diet for rearing fire ant colonies in the laboratory.

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