Red imported fire ant (*Solenopsis invicta* Buren) bait matrix evaluation protocol: Development of a new attractive/palatable all-season fire ant bait

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Bait formulated insecticide is a cost-effective and environmentally sound insecticide formulation for control of the red imported fire ant, *Solenopsis invicta* Buren (Hymenoptera: Formicidae) (Williams et al. 2001). The objectives of this study were to: 1) compare feeding preferences of fire ants towards different bait matrices in laboratory study; 2) observe initial (5 min –1 hr) behavioral preferences like visits, arrestment, and active recruitment towards the bait matrices; and 3) measure the consumption of different matrices overtime. No active ingredients were involved in these assessments.

Materials and Methods

Six *S. invicta* colonies were collected on Feb. 6, 2008 from the USDA Pecan Genetics laboratory grounds in Brazos County, TX. Ants were separated from soil by drip flotation on Feb. 7 & 8, 2008. They were removed and placed in plastic trays measuring 27 x 37 cm x 9 cm tall with inner vertical surface coated with Fluon®, a teflone-like substance, to prevent ant escape. One Petri dish, (14 cm dia. x 2.5 cm tall) contained Castone® that was moistened with water, and had a lid in which holes were drilled to allow ants to enter and exit, was placed in each colony tray to house the queen, brood and worker ants. Each colony was provided with water, and maintained under standardized laboratory conditions. Each laboratory colony served as one replication. These six colonies were used to conduct three consecutive trials, thereby increasing the number of replications to 18, so that results could be analyzed either for each individual trial or for the three trials collectively. A HOBO® H8 Pro Series Data Logger (Onset Computer Corp., 470 MacArthur Blvd., Bourne, MA 02532, (www.onsetcomp.com) was used to record temperature and humidity throughout the course of these trials.

Colonies used in these trials were assessed before and after each trial period for colony vigor and the number of queens, alates, workers and brood were recorded so it could be determined if any definitive trends would occur due to conditions under which matrices were offered, or if the ants might not be hungry. Prior to exposing ants to bait matrices, the ants were deprived of food, and were only provided with water for varying periods of time. In Trial 1, food was deprived for 24h; Trial 2 was for 72h; and Trial 3 was for 94h. This was done to insure hunger in the ants.

Five bait matrix formulations were evaluated:

1. X79A-08 – granular bait formulation; dark in color and dense in appearance
2. X80A-08 – granular bait formulation; light yellow and lightweight particles
3. X81-08 – granular bait formulation; tan and moderately dense in appearance
4. X82-08 – gel bait formulation; light orange in color
5. X83-08 – gel bait formulation; light orange in color

Each set of five candidate bait formulations, arranged in random order within each of the six laboratory colony replications (**Fig. 1**) was offered to each individual colony in a foraging arena (a separate plastic tray from the container housing the ant colony) connected to the colony tray by a metal flashing foraging "bridge" (**Fig. 2**). Aliquots of 0.25gm of each bait matrix were weighed in VWR™ International Hexagonel 4.5 cm polystyrene weighing dishes. In each of the foraging arenas, weighing dishes were randomly placed in a circle with each matrix approximately 70^0 to the other **(Fig. 3 & 4**).

At the end of each trial, remaining bait was weighed and the amount removed from the initial 0.25g provided was calculated. Any gain in weigh possibly due to added moisture or weighing error was assumed to be zero loss. Appropriate controls (a set of bait matrix formulation aliquots weighed and set aside to determine if weigh loss occurred in the absence of ant foraging) were not used in Trial 1. In Trials 2 and 3, replicates of each matrix (0.25g) were held (excluding ants) in large Petri dishes with lids removed (14cxm x 2.5cm deep) along side matrices exposed to ants in colony tray arenas and re-weighed at the conclusion of the trials.

Observations on the number of foraging workers visiting each bait matrix (**Fig. 5**) were recorded every 10 minutes for the first 1 hr; once every hour for the next five hours; and every six hours for the next 48h or until all bait was removed for a treatment. The complete removal of bait from each weighing dish was recorded. Results were analyzed for each trial using analysis of variance (ANOVA) and means were separated using Tukey's Test at $P \leq 0.05$.

Results

Colony status for each of the three trials is presented in **Table 1.** Colonies were collected from an area where the polygyne form of *S. invicta* is prevalent, and queen ants were added prior to the initial trial to assure that each colony had a queen. Thereafter, queen absence during these observation periods was not necessarily an indication that queens were absent. Brood persistence was an indicator of continued egg production (**Table 1**). For Trials 2 and 3, additional worker ants were added and colonies starved prior to trial initiation to increase foraging pressure on bait formulations. Observations for each individual trial are presented below:

Trial 1. Response of ants to bait matrix formulations was generally very slow during the first three hours of exposure to ants. Only a few ants (1-3) wandered over some of the baited dishes, but left quickly without picking up bait. However, at one bait matrix (X80-08, rep 3), 50 ants invaded the dish and were busy moving bait particles. Recruitment of workers continued and all 0.25g bait was removed by 7h. Also, all 0.25g of the bait (X81-08, rep 3) had been removed at this time. Small numbers of foraging ants had visited the x79A-08 bait, rep 3, beginning at 5h post exposure and recruitment had increased to 13 ants foraging at 12h, and all 0.25g had been removed by 12h. Bait matrix x83-08 attracted few ants until 12h when 33 ants gathered in the bait dish in rep 3 and began moving pieces of the extruded bait "noodles" by ants (10-20 ants), removing 0.25 g by 18h. Least preferred bait was x82-08. Only 13 ants

visited the bait at 48h. The ants occurred at irregular intervals showing no pattern of recruitment. It appeared that no x82-08 had been removed.

Trial 2. Ants in this trial began to cross-over the bridges from the colony trays to the trays containing baited dishes more rapidly than in Trial 1; however, the movement toward the baits was generally slow and similar to the response shown in Trial 1. These ants had been deprived of food for 72h prior to starting this trial. After 2h exposure, the ants became more active and began to move bait at a faster rate than in Trial 1 due to the strong recruitment of worker ants. At 3h, the ants showed a definite preference for the granular baits and favored the X81-08 bait; and foraging had increased on the granular baits X79A-08 and X80A-08. Foraging on the extruded gel baits X82-08 and X83-08 was light and few ants were attracted to them for the first 6h exposure; however, the X83-08 bait was more attractive to the ants than the X82-08 bait. The ants had removed all of the X83-08 bait from two of the six replicates at 18h; little or none of the X82-08 was removed. The extruded baits dry out rather quickly, but the ants (15-20) were observed moving bait that was dry to the touch.

Trial 3. The ants were initially slow to respond to any of the baits during Trials 1 and 2. Although the ants in Trial 2 had been deprived of food for 72h prior (to test) to insure hunger, bait particles were still evident in some nesting cells taken in during Trial 1, and ants may have been feeding on these. Prior to starting Trial 3, colonies were deprived of food (given only water) during a 94h period. The ants in this trial responded more readily and within 20 min. of exposure, some ants had visited at each of the baits. Within 60 min., recruitment of worker ants was strong to all baits except X83-08 gel bait. Ants moved bait at a much faster rate than in Trials 1 and 2. Raw data showed a trend toward the granular baits being more attractive than the gel baits; however, the hungry ants foraged strongly on the gel baits also in this trial, and appeared to favor the X82-08 over the X83-08 bait. This was terminated after 10 hours and 20 minutes.

Ant foraging numbers on baits in Trials 1 -3. Overall, there was a trend, with more ants being associated with bait matrix formulations X80A-08 and X81-08 (**Fig. 6**). Ant numbers associated with all bait matrix formulations in weighing trays declined over time (after 6 h) due to removal of bait. Statistical analysis of data from all three trials resulted in no significant differences between the number of ants foraging on bait matrix formulations except at 36 h after exposure: X79A-08 - 1.33a; X80A-08 - 0.67ab; X81-08- 0.42ab; X82-08 - 0.67ab; X83-08 - 0.00b (d.f. = 4; F = 2.821; Mean Sq. = 0.998; P = 0.034). By that time Trial 3 had been terminated, reducng the number of observations. Furthermore, the rate of foraging ant response varied greatly between the three trials because additional worker ants were added and colonies were starved as trials progressed – even though the same six colonies were used for the three replicate trials. This rate of response difference, and because Trial 3 was terminated earlier, increased variability that may have prevented documenting significant differences between mean numbers of foraging ants.

Bait removed in Trials 1 - 3. The total removal of candidate bait matrix 0.25g aliquots from weighing dishes over time was the most direct method of quantifying bait preference, even without performing statistical analysis on the data. The granular bait formulation X80A-08, followed by X81-08, was removed from more dishes over time than were gel baits, with X83-08 being removed more than X82-08 (**Fig. 7**). The granular bait formulation X79A-08 was least removed. This trend was supported, in part, by post-trial weight of remaining bait (**Fig. 8**), with X80A-80 being the most removed (and assumed consumed) and X79A-08 being the least removed. No significant differences were found between bait matrix formulations: X79A-08 - 0.0378g; X80A-08 - 0.1011g; X81-08 - 0.0722g; X82-08 - 0.0783g; X83-08 - 0.0944g (d.f. = 4; $F = 1.357$; Mean Sq. = 0.008; $P = 0.256$).

Minor weigh changes were recorded during the course of Trials 2 and 3 for some of the bait matrix formulations, particularly the gel baits: Trial 2, X79A-08 – 0.00g a; X80A-08 – 0.00g a; X81-08 - +0.03g a; X82-08 – -0.11g b; X83-08 - +0.01g a (d.f. = 4; $F = 36.389$; $P = 0.000$); Trial 3, X79A-08 – 0.00g; X80A-08 – 0.00g; X81-08 – 0.00g; X82-08 – 0.12g; X83-08 - +0.05g.

Discussion

In this trial, “beads” of gel bait were extruded from their applicators into the weighing dishes, producing large particles that were less attractive to the foraging ants and more difficult for them to remove from the tray. Comparing bait removal between granular baits and gel baits as attempted here may not provide useful results. If attractiveness and removal of bait matrix formulations by foraging red imported fire ants involved the influence of particle size and weight, results may have been improved by cutting the “beads” into fine segments similar to bait particle sizes.

Comparisons between granular bait matrix formulations perhaps better reflect the influence of “taste” because of more similar physical characteristics, although X-79A-08 seemed to be the heaviest and densest – and least attractive. The formulation of X80A-08 seemed to be the lightest and appeared similar to conventional ant bait formulations that use processed, de-fatted corn grit as a carrier with soybean oil (containing the active ingredient) as an attractant. This formulation seemed to be the most preferred by the laboratory colonies used in these trials.

However, removal of bait particles from weighing dishes does not constitute ingestion of the particles or whatever is extracted from them by foraging ants, or document the ability of ants to feed the ingredients to other members of their colony through trophalaxis. Other methods, such as use of rare earth elements, dyes or bioassays of active ingredients are necessary to determine which of these candidate formulations will be the best delivery vehicle for applying an insecticide treatment to a red imported fire ant colony.

Literature cited

Williams, D. F., H. L. Collins and D. H. Oi. 2001. The red imported fire ant (Hymenoptera: Formicidae): an historical perspective of treatment programs and the development of chemical baits for control. Amer. Entomol. 47: 146-159.

Table 1. Assessment of laboratory red imported fire ant colonies used in trials evaluating attractiveness of MGK bait matrix formulations, Brazos Co., TX, Feb. 2008.

Trial 1, Feb. 13-15

Pre-treatment Trial 1	**Queens***	**Alates**	**Workers**	Brood **Worker**	present **Sexual**
Colony 1	1	0	30,000	1/8 tsp larvae, pupae	0
Colony 2	1	0	30,000	1/4 tsp larvae, pupae	0
Colony 3	1 physogastric	0	35,000	1/4 tsp larvae, pupae	0
Colony 4	1	0	35,000	1/2 tsp larvae, pupae	0
Colony 5	1	40 males; 10 females	25,000	1/8 tsp larvae, pupae	0
Colony 6	1	15 males; 5 females	25,000	1/8 tsp larvae, pupae	0
Post-treatment Trial 1					
Colony 1	0	0	30,000	1/8 tsp pupae	0
Colony 2	0	0	30,000	1/4 tsp pupae	0
Colony 3	0	0	35,000	1/4 tsp pupae	0
Colony 4	0	0	35,000	1/4 tsp pupae	0
Colony 5	0	40 males; 10 females	25,000	1/8 tsp pupae	0
Colony 6	0	0	25,000	<1/8 tsp pupae	0

* One queen was added to each colony to insure queen presence

Trial 2, Feb. 18-20

Pre-treatment* Trial 2	**Queens**	**Alates**	**Workers**	Brood **Worker**	present **Sexual**
Colony 1	0	0	30,000	1/8 tsp pupae	0
Colony 2	0	0	30,000	1/4 tsp pupae	0
Colony 3	0	0	35,000	1/4 tsp pupae	0
Colony 4	0	0	35,000	1/4 tsp pupae	0
Colony 5	0	40 males; 10 females	25,000	1/8 tsp pupae	0
Colony 6	0	0	25,000	<1/8 tsp pupae	0
Post-treatment Trial 2					
Colony 1	0	0	30,000	1/8 tsp pupae	0
Colony 2	0	0	30,000	1/8 tsp pupae	0
Colony 3	1	0	35,000	1/4 tsp pupae	0
Colony 4	0	0	35,000	1/4 tsp pupae	0
Colony 5	0	40 males; 10 females	25,000	1/8 tsp pupae	0
Colony 6	0	0	25,000	<1/8 tsp pupae	0

* About 5,000 ants were added to each colony pre-treatment, and the trial was initiated after a 48 h period of starvation.

Trial 3, Feb. 18-20

Pre-treatment* Trial 2	**Queens**	**Alates**	**Workers**	Brood **Worker**	present **Sexual**
Colony 1	0	0	30,000	1/8 tsp pupae	0
Colony 2	0	0	30,000	1/4 tsp pupae	0
Colony 3	1	0	35,000	1/4 tsp pupae	0
Colony 4	0	0	35,000	1/4 tsp pupae	0
Colony 5	0	40 males 10 females	25,000	1/8 tsp pupae	0
Colony 6	0	0	25,000	<1/8 tsp pupae	0

Post-treatment Trial 2**					
Colony 1	0	0	30,000	1/8 tsp pupae	0
Colony 2	0	0	30,000	1/4 tsp pupae	0
Colony 3	0	0	35,000	1/4 tsp pupae	0
Colony 4	0	0	35,000	1/4 tsp pupae	0
Colony 5	0	40 males 2 females	25,000	1/8 tsp pupae	0
Colony 6	0	0	25,000	< 1/8 tsp pupae	0

* About 5,000 ants were added to each colony pre-treatment, and the trial was initiated after a 48 h period of starvation.

** Colonies were not fed from Feb. 20, 11:00 a.m., to Feb. 25, 94h prior to starting the trial

Fig, 1. Bait matrix trial replications

Fig. 2. Bait matrix trial laboratory colony and foraging arena

Fig. 3. Bait matrix trial with *S. invicta* foraging workers crossing bridge to 0.25 quantities of bait

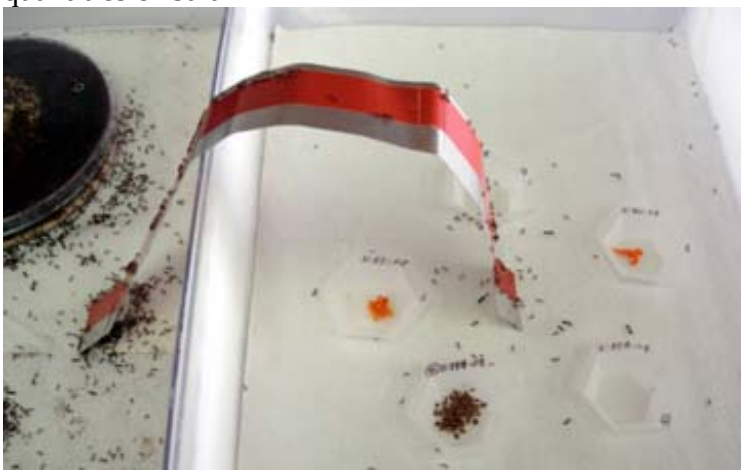


Fig. 4. Bait matrix arrangement, an example of randomized placement of five candidate formulations

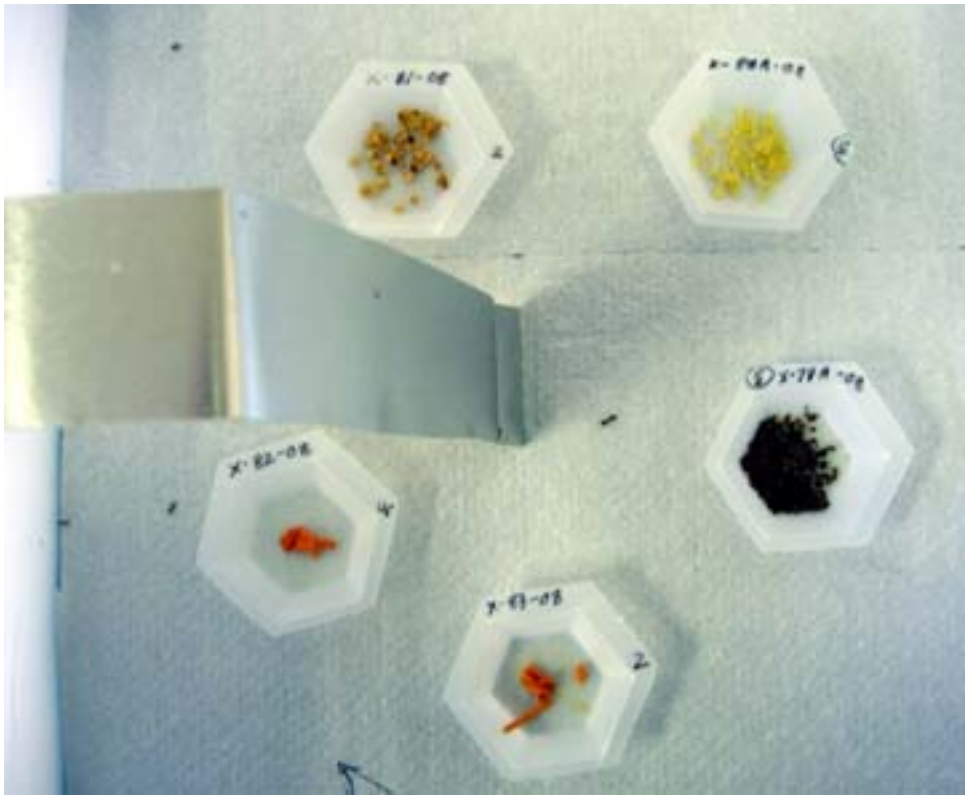


Fig. 5. *S. invicta* foraging workers associated with candidate bait formulations

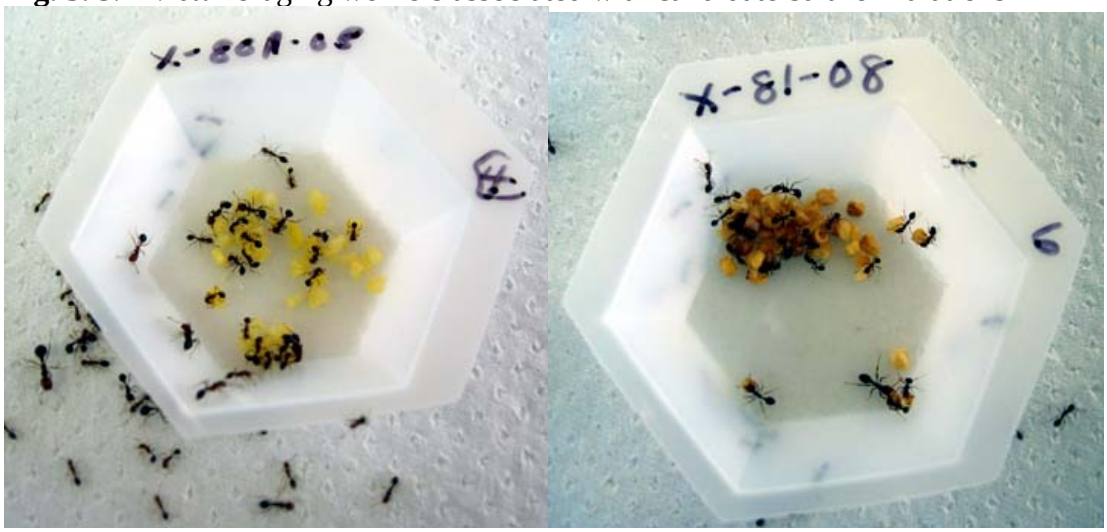


Fig. 6. Mean number of foraging red imported fire ant workers associated with bait matrix formulations in three trials of six laboratory colonies per trial, Brazos Co., TX, Feb. 2008.

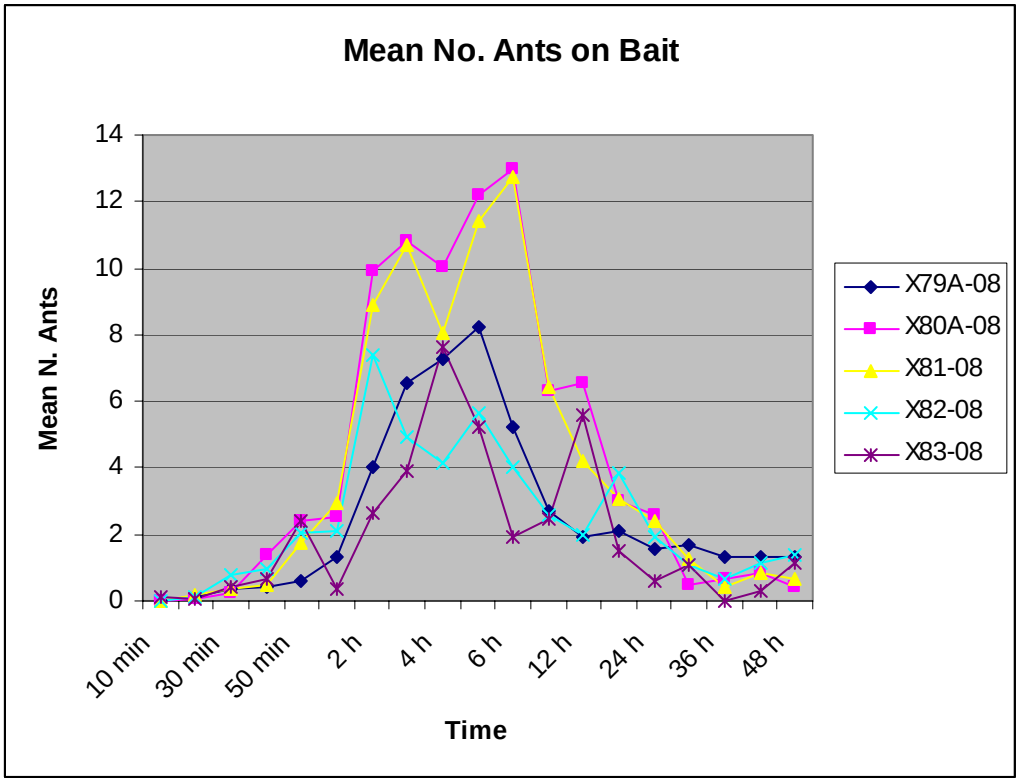
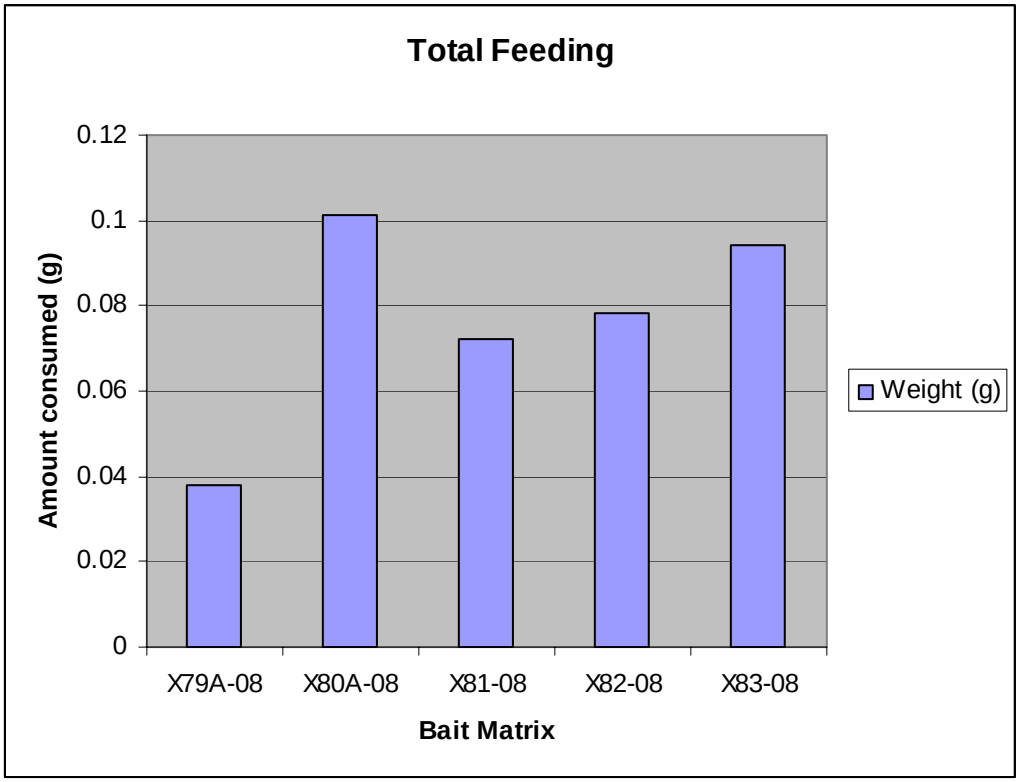


Table 7. Number of empty weigh dishes over time from three trials of six laboratory colonies per trial, Brazos Co., TX, Feb. 2008.

Fig. 8. Mean amount bait of 0.25 g removed by foraging fire ants in three trials of six laboratory colonies per trial, Brazos Co., TX, Feb. 2008.



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