Incidence of Feeding by the Eastern Subterranean Termite (Isoptera: Rhinotermitidae) in Laboratory Bioassay.

by

Brian T. Forschler[1]

ABSTRACT

Eastern subterranean termite workers were fed cellulose mixed with a fluorescent pigment, in a series of bioassays, to assess the incidence of feeding. The fluorescent pigment was retained within the alimentary tract and was visible through the insects cuticle. Termites that were provided pigmented cellulose *ad libitum* for 10 consecutive days showed that 55.6%±14.4 (range 16-96%) of the termites had pigmented alimentary tracts on any given day. In tests where termites were provided pigmented cellulose for 24h then removed to feed on unpigmented cellulose, 26.6% retained pigment in the alimentary tract 48h later and 4.8% after 72h. In transfer assays, pigment was transferred to 7% of the recipient termites after 24h. The incidence of individual termite feeding over a 7 day period varied with 9% of the termites never showing pigment and 56% showing continually pigmented alimentary tracts. These tests demonstrate that the incidence of feeding by individual termites is not predictable and the implications of this feeding behavior on termite bait efficacy is discussed.

INTRODUCTION

Eusocial insects present special challenges to researchers attempting laboratory behavioral assays. Survivorship and behavior of termites in laboratory assay can be affected by humidity, temperature, container size, population density, matrix material, and colony vigor (Haverty & Nutting 1974, Esenther 1977, Lenz & Williams 1980, Su & La Fage 1984). One hundred or more termites per replicate are not unusual in laboratory experiments (Smythe & Carter 1970, Su & LaFage 1984, Delaplane & La Fage 1987). Therefore, assays of termite feeding rates are complicated by the fact that numerous individual animals are required in each replicate to assure acceptable survivorship over the course of the test. Identifying individuals within groups that have fed is not easy. One solution is to use radioactive trace elements incorporated into the food substrate (McMahan 1969, Beard 1974). Another technique involves dyes which are transferred to the fat

[1]Department of Entomology, University of Georgia, Georgia Experiment Station, Griffin, GA 30223

body and can be seen through the translucent cuticle of the termite (Su et al. 1994).

Recent interest in the use of toxic baits for control of subterranean termites has raised the question of measuring bait toxicant efficacy. Su et al. (1994) provided small groups of termites with a limited amount of toxicant-treated food containing a fat soluble dye to identify those insects that ingested toxicant for inclusion in bioassay. However, the dye consumed in one feeding episode can result in dye deposition which remains with that individual until that fat body has been replaced. As a result, fat soluble dyes cannot be used to indicate the incidence of individual feeding episodes. It is generally assumed that not all termites feed in equal amounts every day, yet this question has not been pursued to determine the proportion of individuals which feed or have food in their alimentary tract. This paper reports results from studies conducted using a fluorescent dye which is retained in the alimentary tract to determine the incidence of feeding by individuals in groups of *Reticulitermes flavipes* (Kollar) termites.

MATERIALS AND METHODS

Termites.

Termites were collected from infested logs found at the Westbrook farm in Spalding County, Georgia. Logs were returned to the laboratory and termites removed using the technique of La Fage et al. (1983). Termites retrieved from logs were maintained in plastic boxes containing moistened filter paper and pieces of pine wood (*Pinus* sp.) in total darkness at 24°C for at least two weeks before inclusion in bioassay. Only worker caste termites of third are greater instar were used in all tests. Species were identified by alates associated with each group of termites (Weesner 1965).

Pigment and Food Substrate.

Termite feeding substrate was prepared by mixing 1 gm of cellulose powder (Whitmire Research Laboratories, St. Louis, MO) and 0.03g of Blaze Orange fluorescent pigment (DAYGLO Color Corp., Cleveland, OH) moistened with 1.5 ml of distilled water. Controls received cellulose powder moistened as described for the pigmented substrate. Approximately 100 termites were placed in a 100 X 60mm Petri dish containing 6g of sand moistened with 2ml of distilled water and approximately 0.3g of the orange-pigmented cellulose or unpigmented cellulose only. Termites which had fed on pigmented cellulose were identified by the orange coloration of their alimentary tract which was visible through the insects cuticle. The intensity and position of the

coloration also was recorded. However, termites were observed with either a deep orange color throughout the alimentary tract, a lighter coloration of the entire alimentary tract or with pigment visible only in the rectum. Because the intent was to sample each replicate non-destructively and because of the subjective nature of the light versus dark pigmentation scores, termites with any alimentary tract pigmentation or rectum only pigmentation were scored separately. The presence of pigment in termite alimentary tracts was recorded in a darkened room under UV light at 365nm wavelength (Ultra-Lum model UVAC-16, Ultra-Lum Inc., Carson, CA).

Percentage of termites with pigment in alimentary tracts.

Tests were conducted to determine the percentage of termites within a group that had pigmented alimentary tracts indicating that they had recently fed. In these tests 25 termites were placed in a Petri dish and provided pigmented cellulose *ad libitum*. The number of termites with orange alimentary tracts was recorded every 24h for 10 days. There were 6 replicates from 3 source colonies.

Retention time of pigment in the alimentary tract.

Twenty-five termites were placed in Petri dishes with pigmented cellulose as previously described. Pigmented cellulose was replaced with unpigmented cellulose after 24h. The number of termites with pigmented alimentary tracts was recorded every 24h for 4 days. There were 28 replicates from 9 source colonies.

Transfer of pigment.

Two hundred termites from each of 2 source colonies were placed on pigmented or unpigmented cellulose. Those that were placed on unpigmented cellulose were topically marked with green paint. After 24h, 10 termites that showed pigmented alimentary tracts were removed by allowing them to crawl onto a piece of filter paper and gently transferred to a Petri dish containing only unpigmented cellulose along with 10 paint-marked individuals from the same source colony. Every 24h for 3 days the number of paint-marked and unpainted termites which showed pigment in the alimentary tract was recorded for each of 7 replicates.

Presence/absence of pigment in individual termites over time.

Tests designed to examine presence/absence of pigment in individual termites over time were conducted by placing 25 termites into a Petri dish with pigmented cellulose as described for the previous tests. After 24h, seven individual termites within a Petri dish which displayed orange pigmented alimentary tracts were topically marked

Table 1. Mean (±SE) and median percent of termites, by day, showing pigment in the alimentary tract from termites continually exposed to pigmented cellulose for ten consecutive days.

	Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7	Day 8	Day 9	Day 10	Mean
mean	53.3	50.7	51.1	53.0	63.0	55.8	50.2	65.0	66.2	66	55.6
±SE	18.9	10.0	8.4	9.3	10.2	7.4	10.6	17.6	17.2	18.7	15.4
median	56	48	50	54	62	55	62	61	66	70	56

with one of seven different colors for referenced identification. In an equal number of Petri dishes, the termites that were paint-marked were those which did not show orange pigment after the first 24h exposure. Termites were marked using Odds 'n' Ends fast dry enamel (Plasti-Kote Co., Inc, Medina, OH) by taking a blunted #2 insect pin dipped in paint and touching the head or thorax of a termite. The seven colors used were, white (B4), pink (B14), green (B9), red (B13), blue (B23), yellow (B11), purple (made by mixing blue and red), and orange (made by mixing red and yellow).

The presence or absence of pigment in each paint-marked individual was recorded every 24h for three separate tests of 7-day, 8-day, or 12-day duration. If a paint-marked individual died or was cannibalized at any time during these tests the data collected on that individual were not included in the analysis. Only paint-marked individuals which were found throughout each bioassay were included.

There were 5 source colonies used for 11 replicates for the 7-day and 8-day tests and 8 replicates for the 12-day test which ultimately included 55, 52, and 34 individual termites respectively. Half of the aforementioned replicates included individual termites which had pigment in the alimentary tract after 24h and the other replicates contained termites which showed no pigment after the first 24h.

RESULTS AND DISCUSSION

Percentage of termites with pigment in alimentary tract.

The mean percentage of termites with pigment in the alimentary tract was 55.6 ±14.4% (range 16 - 96%) on any given day over a 10 day period (Table 1). There was considerable variation in the percentage of termites showing pigment between replicates as indicated by the standard errors for the means which ranged from 13 to 35% of those means (Table 1). These data underscore the variable nature of termite feeding behavior.

Retention time of pigment.

Twenty-four hours after removing the termites from pigmented cellulose, over half of the termites (60±25%, range 18-100%) retained

Table 2. Summary of laboratory tests where termites were continually exposed to pigmented cellulose for 7, 8, or 12 days.

Test Duration (in days) and Number of replicates	% of termites which showed alimentary tract pigment throughout test	% of termites which never showed alimentary tract pigment throughout test	% of termites which showed pigment for 7 consecutive days	% of termites which did not show pigment for 7 consecutive days	Mean±SE number of cycles of pigment and no pigment
7 days n = 55	20	5	20	5	2.27±1.11
8 days n = 52	33	6	40	6	2.27±1.42
12 days n = 34	3	0	56	9	3.76±1.49

pigment and all replicates still had termites showing pigment in the alimentary tract. Forty-eight hours after removal from treated cellulose 36% of the replicates provided at least one termite with orange pigment in the alimentary tract with 26.6± 24.4% of the original number still showing pigment across all replicates. Only 4.8 ±12.3% of the original number were showing pigment by 72h and only 18% of the replicates provided at least one pigmented individual. None of the termites had pigment in their alimentary tracts 4 days after removal from pigmented cellulose.

These tests showed that 40% of the *R. flavipes* workers tested voided their alimentary tract of food consumed in one day within 48h while one third maintained food in the alimentary tract for two days. This is in contrast to studies with other termite species which indicate a 24 h passage for food through the alimentary tract (La Fage & Nutting 1978). The 5% that showed pigment at 72h could have been termites that received pigment through transfer from other termites after removal from the pigmented food source.

Transfer of pigment.

After 24h, 8.6% (n=70) of the termites from the unpigmented cellulose treatment gained pigment. The percentage of termites not fed pigmented cellulose that showed pigment at 48h was 7.1%. No termites from either treatment regime showed pigment in their alimentary tract by 72h. After 24h, each Petri dish was spotted with pigmented fecal material. Therefore, it was not possible to determine if the source of pigment in the untreated individuals was from direct

Table 3. List of consecutive days with and without pigmented alimentary tracts for individual termites continuously exposed to pigmented cellulose for 12 days.

termite number	days in a row with pigment	days in a row without pigment	days in a row with pigment	days in a row without pigment	days in a row with pigment	days in a row without pigment	days in a row with pigment
1	1	1	10				
2	4	1	7				
3	2	5	4	1			
4	6	6					
5	2	1	9				
6	3	1	8				
7	2	1	2	7			
8	2	1	9				
9	1	2	1	2	6		
10	4	1	7				
11	1	1	7	1	2		
12	1	1	6	4			
13	2	1	1	1	7		
14	3	1	8				
15	1	1	10				
16	2	1	1	2	1	1	4
17	12						
18	1	7	4				
19	6	1	5				
20	3	1	8				
21		1	11				
22		1	2	3	1	4	1
23		1	11				
24		1	11				
25		1	1	3	7		
26		1	2	2	5	1	1
27		5	4	1	1	1	
28		1	1	1	8	1	
29		1	2	9			
30		1	7	1	1	1	1
31		4	4	1	1	1	1
32		5	7				
33		1	2	2	5	1	1
34		6	5	1			

trophollactic transfer or coprophagy. This bioassay also indicates the complete passage of food through the alimentary tract in *R. flavipes* workers occurs within 48h.

Presence/absence of pigment in individual termites.

Certain individuals feed more frequently than others and there was great variability in pigment retention times. Individual termites went through at least one cycle of showing pigment and not showing pigment every 7 days (Table 2). Depending on the duration of the test, 5 to 9%

of the termites tested showed no pigment for 7 consecutive days (Table 2). In contrast, 20 to 56% of the termite workers showed pigment in the alimentary tract for at least 7 consecutive days (Table 2). The 12 day tests showed the average number of consecutive days a termite kept pigment in the alimentary tract was 4.14 ±9.0 days (Table 3). From the same tests, the mean number of consecutive days termites spent without pigment was 2.03 ±1.93 days (Table 3). The breakdown of consecutive days individual termites showed pigment or no pigment illustrates the variable nature of termite feeding episodes (Table 3).

SUMMARY

These results raise questions concerning application of termite baiting technology and selection of termite bait active ingredients (AI's). Although the amounts of pigmented cellulose eaten were not quantified in therse tests, the variability in the feeding cycles demonstrate that the onset of symptoms from a termite bait AI may be expressed differentially by individual *R. flavipes* workers (Table 3). The time to expression of lethal effects also could be affected because ⅓-½ of the termites within a group are likely to have food in their alimentary tract for 7 consecutive days whereas others feed on a less predictable schedule (Tables 2 & 3). In addition, pigment retention assays demonstrated that 40% of the termites passed food through the alimentary tract one day before other termites that fed on the same day.

The time to expression of toxic effects is often dependent on the amount of AI ingested. The individual feeding cycles, recorded in these assays, presents a challenge to the use of an AI that shows a steep dose/mortality curve. These data suggest that a termite AI should have a shallow dose/mortality curve to allow for the variable individual feeding rates. If the AI produces sudden onset of toxicity it will inevitably kill only a portion of the individuals exposed to the termite bait, effect sublethal responses in others, and provide no effects on the rest.

The intent of any bait control strategy against termites requires a toxicant with sufficiently slow expression of lethal effects to allow transport of the toxicant by foragers thourghout the colony. Therefore, an additional question is raised by the transfer of pigment tests. If toxin transfer among termites is similar to the pigment transfer observed in these studies then it may require actual visitation to the bait site by a majority of the colony to produce considerable mortality within a termite colony. If termites do not move between feeding sites on a daily or weekly basis, those termites which are feeding more frequently are likely to be the first to die and will die at the baiting site. I have observed this in laboratory tests with the AI's abamectin and zinc borate hydrate.

If termites die at the bait site, other live termites are likely to avoid that bait site thereby futher reducing the number of termites which could obtain a lethal concentration of AI.

Therefore, a termite bait AI must produce mortality within the framework of the incidence of feeding and rotation of termites to the bait matrix to effect a majority of the colony members. Determining that rate will require extensive information on termite seasonal feeding activity and movement between feeding sites from field studies.

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