A Dye, Sudan Red 7B, as a Marking Material for Foraging Studies with the Formosan Subterranean Termite[1, 2]

by

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ABSTRACT

Workers of *Coptotermes formosanus* Shiraki were fed filter paper stained with Sudan Red 7B. The dye was ingested and the amount of dye absorbed was measurable using a spectrophotometer. This dye was not transferred from individual to individual by trophallaxis at a significant level. The amount of dye remaining in termite tissue after 9 days of fasting was directly proportional to the total number of days the termites were allowed to feed on stained filter paper, regardless of the number of days between feedings.

INTRODUCTION

Due to the size of their colonies, remedial control of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, has been extremely erratic and unsatisfactory. These problems are in large measure due to the necrophobic behavior of this termite (Su 1982, Su et al., 1982). One method of remedial control might be to expose termites to a slow acting insecticide, which would allow the foragers to carry the insecticide back to the entire colony, recruit other termites to the feeding site and/or feed the insecticide to nestmates by trophallaxis. To utilize such a slow-acting insecticide in remedial control, we need to more fully understand the foraging behavior of this termite. Due to their cryptic habits, studying

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[1]*Coptotermes formosanus* Shiraki (Isoptera:Rhinotermitidae)

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foraging behavior of *C. formosanus* requires a marking material.

To date, most workers studying foraging behavior of suberranean termites have utilized radioactive tracers as markers. Gosswald and Kloft (1963) used ^{32}P to study the food exchange of termites in small colonies in the laboratory. Although their techniques were adopted by many researchers, subsequent investigations have concentrated on the food transfer among the nestmates of termites (McMahan, 1963, 1969; Alibert, 1963, 1965). The techniques have been extended to trace the subterranean activity of termites (Spragg and Fox 1974), and to identify which individuals or castes are found at feeding sites (Spragg and Paton 1980).

Few studies have examined the foraging of *C. formosanus*. King and Spink (1969) first studied foraging of *C. formosanus* by digging and following galleries from the central nest. Li et al. (1976), utilizing a ratioactive ^{131}I, discovered that *C. formosanus* could forage as far as 52.1 m from the main nest, and that foraging activity reached the peak at 1 to 3 a.m. and was influenced by storms. Lai (1977) reported that a colony of this termite can range as far as 110 m in Hawaii, which was approximately equivalent to the results reported by Li et al. in China.

Radioisotope techniques have been preferred by many researchers for foraging studies; however, handling of these materials always involves safety problems and requires licensing of the investigator. A dye, Sudan Red 7B (BASF Wyandotte Co., NJ), was selected as a candidate marking material to examine the foraging activity of *C. formosanus*. This dye was similar to that utilized by Lai (1977) to estimate the population of this termite. Any marking material used to trace the subterranean activity of *C. formosanus* must remain in the termite during the experimental period and ideally must be measured quantitatively. This study was initiated to examine the nature of Sudan Red 7B when fed to *C. formosanus* and to evaluate its potential for use in foraging studies.

MATERIALS AND METHODS

Transfer of Dye by Trophallaxis

Termites were collected from a field colony, using a trapping technique described by Tamashiro et al. (1973). Five hundred workers, undifferentiated individuals beyond the 3rd instar, were introduced into a petri dish in which a moistened piece of filter paper stained with 4% (w/w) Sudan Red 7B was placed. This unit was placed in an incubator at 30°C for 14 days. At the end of 14 days all of the workers had eaten some stained filter paper and had become stained themselves. After this period, 200 stained workers along with 200 workers which were fed on untreated filter paper were placed into a petri dish containing moistened, unstained filter paper. At 5, 10, 15, 20, 25, and 30 days, the number of stained individuals was counted.

A Chi-square test was used to determine if the ratio was significantly different from 50%. Each observation period was replicated 10 times.

Quantitative Measurement of Dye Ingested by Termites

Two pieces of filter paper containing 4% (w/w) Sudan Red 7B were placed in a petri dish. The papers were moistened, then approximately 200 field collected workers were placed in the petri dish. A total of 48 such units was prepared and stored at 30°C. Four units were selected daily, at random, for up to 12 days. From each of the four randomly selected units, 15 individuals were selected at random, weighed (wet weight), and homogenized in a grinder with 3.6 ml acetone. The homogenized solutions were centrifuged at 15,000 rpm for 15 min. The amount of dye in the termites was measured with a spectrophotometer (Beckman^{R}, Model 26). The absorbance of the supernatant was read at a wave length of 450 nm. The unit absorbance was obtained by converting the actual absorbance into that per one gram termites in one ml acetone solution.

Thus, $A = 3.6\ A'/W$

Where: A = unit absorbance,

A' = actual absorbance read from spectrophotometer,

W = weight of 15 termites tested.

After the first 15 individuals were removed, termites which had been continuously exposed to stained paper for 1, 3, 6, 9, and 12 days were transferred to petri dishes containing unstained paper. At 1, 2, 5, 8, 11, 14 and 29 days after transfer, 15 termites were selected at random from each unit of each treatment, and the absorbance of the dye extracted from each group of 15 termites was obtained by the same procedure described above. There were four replications for each of these treatments.

Continuous Feeding and Intermittent Feeding

Measurements of dye absorption described above were based on termites continuously feeding on stained filter paper. However, continuous feeding is unlikely to occur under field conditions. A termite may feed on unstained natural food for a certain period after feeding on the stained materials, then go back to feed on stained materials. Thus, to be able to apply the measurements obtained from continuously feeding experiments to field studies, it was necessary to examine whether there are differences in absorbances for termites fed on stained materials continuously and those fed intermittently.

Five hundred field collected workers were introduced into a petri dish with two pieces of filter paper stained with 4% (w/w) Sudan Red 7B. Eighteen units were prepared and stored at 30°C. Nine units were assigned for 2 days of accumulated exposure, the remaining were assigned to 3 days of accumulated exposure. Of the nine units in each exposure time, three units were assigned for continuous exposure, three for 2-day and three for 4-day intervals of feeding on unstained paper. Thus, there were six treatments with three replications for each treatment. Nine days after the last day of feeding on stained papers, the absorbance of 15 individuals selected at random from each treatment was examined as described above. The absorbances of 6 groups (replications) of 15 termites were evaluated for each treatment. Results were subjected to analysis of variance using a completely randomized design. Significantly different treatment means were statistically separated at the $P = 0.05$ level by Duncan's multiple range test.

RESULTS AND DISCUSSION

Transfer of Dye by Trophallaxis

The dye apparently was not passed on from stained to unstained individuals by trophallaxis (Table 1). If exchange of dye had occurred, the

Table 1. Percentage of stained termites at various time intervals after 200 stained and 200 unstained termites were mixed in a petri dish.

Days after mixing	5	10	15	20	25	30
Percentage of termites stained	49.8	49.2	49.4	45.3*	43.1*	42.7*

* Chi-square test indicates percentage significantly less than 50% at P = 0.05 level.

number of stained termites would have increased to more than 50%. Instead, there was a gradual decrease in the percentage of stained individuals. Although the percentage of stained individuals was always below 50%, the Chi-square test showed that there were no statistically significant differences for the first 15 days. The significantly smaller proportion of stained individuals in the last three observations may have been due to excretion or elimination of the stain before it was absorbed or higher mortality in the stained individuals. Because termites used in this experiment were foragers by definition (they were collected from foraging sites), we feel this experiment suggests that no significant amount of this dye was transferred from forager to forager. The dye was only obtained by direct feeding.

Quantitative Measurement of Dye Fed by Termites

Absorbance of dye in termites which were continuously fed on stained filter paper increases steadily for 12 days (Fig. 1). The sharp rise in absorbance during the first few days was probably due, in large measure, to dye contained in the gut. The tapering of absorbance in the later days of the experiment was probably due to the dye in the gut plus the dye in the tissues. Penetration of the dye from the gut into the hemocoel, and subsequently into the tissues, apparently was at a slower rate than the rate of ingestion of the dye. The amount of dye in termites decreases sharply immediately after the termites stopped feeding on the stained paper. This was probably due to the elimination of the dye present in the gut. After the elimination of the dye in the gut, the dye appears to disappear much more slowly from the tissue (Fig. 1).

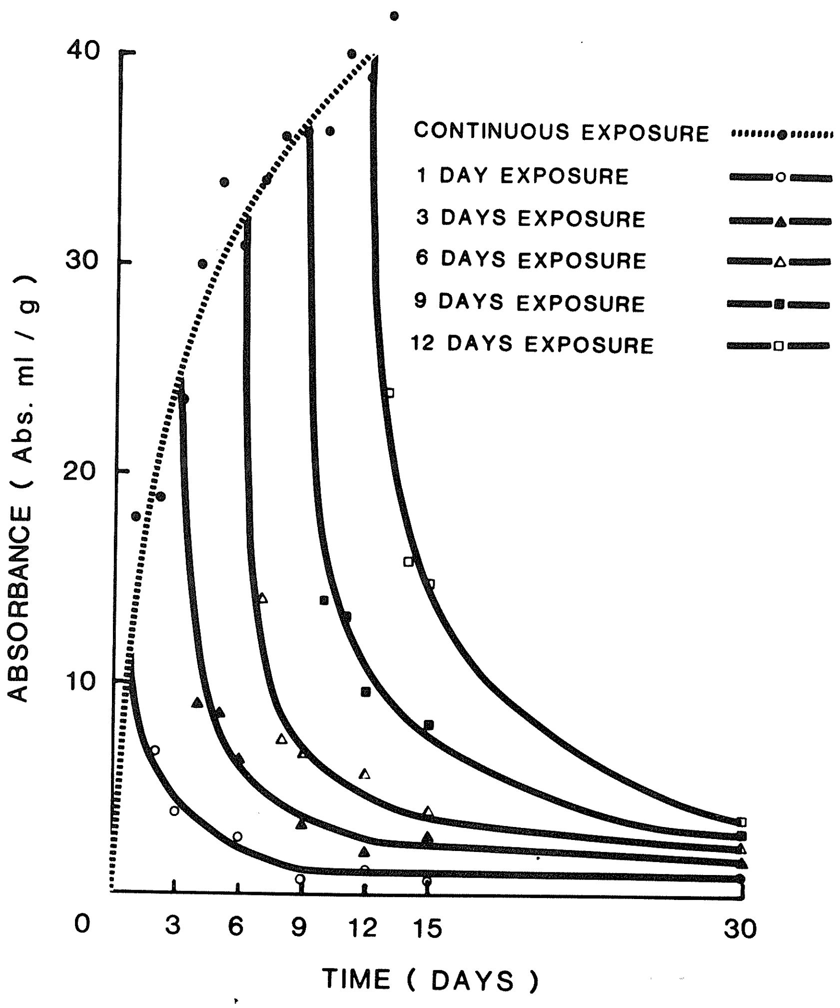

Fig. 1. Absorbance curves at 450 nm for termites fed Sudan Red 7B dye for different durations of time.

In general, the decay curves are described by a negative exponential function. Also, each of these decay curves usually stabilized at a characteristic level 8 to 10 days after the transfer. Termites which had been exposed for longer periods acquired more dye within their tissues, and the decay curves stabilized at higher levels of absorbance. Thus, if the termites fed on stained materials for a certain period then on unstained materials for more than 8 to 10 days, the stabilized absorbances from such termites may be utilized to estimate the amount of food consumed during the periods when termites fed on stained materials.

Continuous Feeding and Intermittent Feeding

There were no significant differences between the amount of dye in termites fed stained filter paper continuously and intermittently for the same total number of days (Table 2). Significant differences were only seen

Table 2. Stabilized absorbances for termites fed stained filter paper continuously or intermittently for 2 or 3 accumulated days with different intervals between feeding.

Accumulated days of exposure	Time interval between feeding on stained filter paper (days)*		
	0	2	4
2	1.44[a]	1.52[a]	1.64[a]
3	2.00[b]	1.82[b]	2.22[b]

* Throughout the table, means followed by the same letter are not significantly different by Duncan's multiple range test at P = 0.05 level.

between treatments with different total accumulated days of feeding. Thus, it was clearly shown that as long as the termites fed on filter paper for the same number of days regardless of whether or not the feeding was continuous or intermittent, absorbances stabilized at the same level. Stabilized absorbance obviously depends upon the total length of exposure rather than the mode of exposure.

Results of this study indicate that Sudan Red 7B was not transferred from forager to forager by trophallaxis, and that when termites were allowed to feed on stained filter paper for a given period then fed unstained filter paper for 8 to 10 days, the stabilized absorbance can be used to indicate the amount of time the termites fed on stained material. Thus, if the stained materials were introduced into foraging sites of a colony of *C. formosanus* for a certain period, and subsequently removed for 8 to 10 days, the stabilized absorbance obtained from a single individual can be used to express the frequency of feeding by that individual forager at the foraging site during the period the stained materials were offered to the colony.

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