Evaluation of the Dye Marker Sudan Red 7B with Reticulitermes flavipes (Isoptera: Rhinotermitidae)

by

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ABSTRACT

The oil-soluble dye Sudan Red 7B, synonymous with Fat Red 7B, is useful for marking subterranean termites (Isoptera: Rhinotermitidae) in field studies, but the appropriate dosage and exposure period must be determined with different termite species. We evaluated dye retention, and effects on feeding and mortality with the eastern subterranean termite, *Reticulitermes flavipes*. As has been found with the Formosan subterranean termite, the dye was not transferred noticeably by trophallaxis. Significant mortality and feeding deterrence were observed during 15 day exposure to 1%, 2%, and 4% dye, and feeding was significantly reduced on papers impregnated with 4% dye even during 3 and 5 day exposure periods. Dye concentrations of 2% or less and 3-5 day exposure periods are appropriate for marking *R. flavipes* for field studies lasting around 15 days.

INTRODUCTION

Study of the population demographics and foraging behavior of *Reticulitermes* species (Isoptera: Rhinotermitidae) poses difficulties, due to the subterranean gallery system and the absence of a well-defined nest architecture that is separable from the surrounding soil matrix. The oil-soluble dye Sudan Red 7B, synonymous with Fat Red 7B, has been used successfully in studies with the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Lai 1977; Su et al. 1984; Su and Scheffrahn 1988a, 1988b). This dye is not passed significantly by trophallaxis (Su et al. 1983b) and its toxicity is concentration dependent (Lai et al. 1983; Su et al. 1983a; Su et al. 1988), although Delaplane et al. (1988) reported feeding suppression even with low dye concentrations. Length of retention of the dye by *C. formosanus* and toxicity increase with dye concentration and length of exposure

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(Su et al. 1983a; Delaplane et al. 1988).

Although Esenther (1980) used dyed termites in a mark-recapture study to estimate the size of several *Reticulitermes flavipes* (Kollar) colonies, little information is available on the effects of marker dyes on *Reticulitermes* species. Su et al. (1988) concluded that low-toxicity concentrations of Sudan Red 7B were not retained sufficiently long by *R. flavipes* for use in field studies when the experimental period was greater than two weeks, and suggested the need for an alternative dye with this species. However, the extensive use of Sudan Red 7B with *C. formosanus* prompted us to evaluate its effects on *R. flavipes* and potential for use in studying the colony demographics of this species in urban areas of Ontario. For field studies during the short summer period of peak termite activity in Ontario, our aim was to identify the minimum dye concentration and exposure period that would clearly label the insects for 15 days, but fade appreciably by 30 days.

MATERIALS AND METHODS

Eastern subterranean termites, *R. flavipes*, were collected in rolled corrugated cardboard within a ca. 10cm ID X 15cm L plastic (ABS) pipe (La Fage et al. 1983) placed on top of an infested maple stump in the City of Scarborough, Ontario, and in similar traps at the surface of the surrounding soil. Termites were maintained in the laboratory in plastic boxes in an unlighted temperature (27±0.5°C) and humidity (95±5% RH) controlled cabinet. Bioassays were also performed in this cabinet. Dye retention and transfer among individuals were measured in polystyrene snap-cap vials (Canlab No. V3001-212, 44.8ml, 60 X 35mm Dia.) containing 7ml (10g) white sand and 1ml de-ionized water; while mortality and feeding were measured in similar vials containing 3ml perlite in addition to the 7ml sand, and 2ml water, capped with a disposable polyurethane foam plug (Canlab No. T1385). The experimental conditions were modified in order to improve matrix moisture retention and increase air exchange within the vials, since fungus growth was noted in some vials in the first experiments.

The insects were fed dyed papers for 3, 5, or 15 days. Experiments were conducted to assess dye retention, dye transfer by trophallaxis, termite mortality, and effects on feeding.

Each dye treatment was replicated three times with groups of 40 *R. flavipes* workers, older than the third instar as determined by size. A strip of Whatman No. 1 filter paper impregnated with a particular dye concentration, or with acetone alone, was placed

along the side of each vial. Solutions of Fat (Sudan) Red 7B dye (Sigma Chemical Co., St. Louis, MO) in acetone were applied to the papers by pipet (1ml on each side of a 9cm Dia. filter paper) to achieve dye concentrations (weight/weight percentage) of 0.5%, 1%, 2%, and 4%. Papers were oven-dried before and after feeding, weighed at a precision of 0.01mg, and mortality and paper weight loss data subjected to analysis of variance (ANOVA, complete randomized design), and significantly different means separated by the Ryan-Einot-Gabriel-Welsch (REGW) Multiple F Test, α=0.05 (SAS Institute Inc. 1987).

To determine whether the dye was transferred by trophallaxis, the number of dyed termites in mixed groups originally consisting of ten dyed and ten undyed workers was recorded at 15 and 30 day intervals. Each time interval was evaluated independently with each combination of dye concentration and exposure period. A one-tail Z test of proportions, α=0.05 (Dixon & Massey 1983), was employed to test whether the proportion of dyed termites in each group exceeded the original 50%.

RESULTS AND DISCUSSION

Workers fed dyed papers containing 0.5% and 1% (wt./wt.) Fat (Sudan) Red 7B for the maximum 15 day period retained their coloration adequately for 15 days, but were still equivalently colored after 30 days (Table 1). Feeding on 2% dye for 5 days, however, provided the desired pattern of dye retention: clear labeling for 15 days, and fading of the dye at 30 days.

Recognition of dyed termites in field collections is a subjective process, affected both by individual visual acuity and lighting (florescent lighting should not be used). Su et al. (1983b) measured dye absorbance after ingestion with a spectrophotometer at 450nm, and we have investigated the use of this technique to determine the presence of dye in captured termites and the amount of time since dye ingestion. However, since collections from a single field trap can contain as few as one dyed termite among ca. 13,000 individuals, spectrophotometry must be applicable to single individuals to be of practical value. Fat Red 7B shows absorbance peaks at 350nm and 506nm, with a valley at 418nm, but high variability among individuals and the extremely small absorbance of individual termites has, to date, prevented us from developing a more objective identification system. Further work in this area is desirable.

When ten dyed termite workers were mixed with ten undyed workers, there were never more than ten dyed individuals recorded in each group 15 and 30 days later, and the proportion of dyed

Table 1. Retention of Fat Red 7B dye in *R. flavipes* workers after feeding on dye-impregnated papers.

Dye Concentration (wt/wt %)	Exposure Period (Days)	Percentage of Clearly Dyed Survivors[a]		
			Post-Exposure[c]	
		Initial[b]	15 Days	30 Days
0.5 %	3	79±8 %	0 %	0 %
	5	77±0	(100)	(45)
	15	97±0	86	80
1.0	3	79±5	0	-[d]
	5	80±5	(100)	(58)
	15	97±3	96	100
2.0	3	97±0	86	0
	5	100±0	100	(100)
	15	100±0	100	-[d]
4.0	3	97±4	92	0
	5	97±0	100	100
	15	100±0	100	-[d]

[a]Percentages are not cumulative. Percentages in parentheses were detectable, but color was too faint to be of practical value in differentiating individuals within large groups of living termites.
[b]N = 3 groups of 40 mixed workers and nymphs.
[c]N = 1 group of 25 mixed workers and nymphs.
[d]No survivors. Fungal growth considered a contributing mortality factor.

Table 2. Changes in the numbers of dyed and undyed *R. flavipes* workers after mixing ten workers fed Fat Red 7B with ten undyed workers.*

Dye Concentration (wt/wt %)	Exposure Period (Days)	Number of Dyed:Undyed Workers		
			Post-Exposure	
		Initial	15 Days	30 Days
0.5 %	3	10:10	0:16	0:18
	5	10:10	9: 9	8: 8
	15	10:10	10: 9	10: 8
1.0	3	10:10	0:19	0: 0
	5	10:10	9:10	7: 9
	15	10:10	9: 9	7: 8
2.0	3	10:10	9:10	0:16
	5	10:10	10:10	9: 8
	15	10:10	7:10	3: 4
4.0	3	10:10	8:10	0:18
	5	10:10	10:10	7:10
	15	10:10	9: 9	6: 9

*Proportions of dyed workers in each group did not significantly exceed the initial proportion of 0.50 (one-tail Z test of proportions, α=0.05).

termites did not significantly exceed the original 50%, regardless of the dye concentration or length of the exposure period (Table 2). This confirmed the absence of any appreciable dye transfer by trophallaxis in *R. flavipes*, as was demonstrated by Su et al. (1983b) with *C. formosanus*. At all concentrations, dye excretion was rapid following the shortest 3 day exposure period.

Significant, and concentration dependent, dye toxicity was noted only over the 15 day exposure period (Table 3). Our preliminary studies indicate that delayed mortality follows this same pattern with respect to dye concentration and exposure time, as has been demonstrated with *C. formosanus* (Su et al. 1983a).

Table 3. Mean (±SD) percentage mortality of *R. flavipes* workers immediately after feeding on papers impregnated with Fat Red 7B.*

Dye Concentration (wt/wt %)	Exposure Period		
	3 Days	5 Days	15 Days
Control	11± 4[a]	21±6[a]	23±1[a]
1%	22± 5[a]	20±9[a]	44±3[a]
2	28±15[a]	16±3[a]	43±5[b]
4	18± 6[a]	26±4[a]	62±5[c]

*N = 3 groups of 40 workers. Means within each column followed by different letters are significantly different (ANOVA, REGW Multiple F Test, α=0.05).

Although no immediate toxicity was apparent over 3 and 5 day exposure periods, termites fed significantly less on papers impregnated with the highest concentration of 4% dye (Table 4). Feeding was significantly less than the control on all dyed papers over the 15 day exposure period. Feeding deterrence or suppression, therefore, should be taken into account if this dye is used to investigate termite feeding preferences. Delaplane et al. (1988) found that *C. formosanus* workers dyed with Sudan Red 7B had fewer intestinal protozoans and continued to feed less than undyed workers, suggesting that the reduced feeding we noted may be due to sublethal physiological effects rather than behavioral avoidance.

In summary, our results, in agreement with those of Su et al. (1988), demonstrate that low concentrations of Sudan Red 7B are rapidly excreted by *R. flavipes*, and that extended feeding periods result in high mortality. Moreover, some feeding deterrence is apparent even over short (3-5 day) exposure periods. However, dye concentrations up to 2% may be used safely to mark *R. flavipes* for short-term studies (ca. 15 days), if the exposure period does not exceed 5 days. Dye marking is a useful

Table 4. Feeding by *R. flavipes* workers on papers impregnated with Fat Red 7B, expressed as the mean (mg±SD) amount of paper removed.*

Dye Concentration (wt/wt %)	Exposure Period		
	3 Days	5 Days	15 Days
Control	10.4±2.2^{a}	10.2±2.1^{a}	39.6±2.7^{a}
1%	7.3±0.7^{ab}	7.7±1.9^{ab}	23.2±5.6^{b}
2	4.5±0.8^{bc}	6.7±1.8^{ab}	20.7±3.7^{b}
4	2.9±1.6^{c}	4.9±1.3^{b}	16.9±1.4^{b}

*N=3 groups of 40 workers. Means within each column followed by different letters are significantly different (ANOVA, REGW Multiple F Test, α=0.05).

technique for field studies, but dosage and exposure time should be recalibrated with different termite species.

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