RETENTION TIME AND TOXICITY OF A DYE MARKER, SUNDAN RED 7B, ON FORMOSAN AND EASTERN SUBTERRANEAN TERMITES (ISOPTERA: RHINOTERMITIDAE)

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ABSTRACT

Workers of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) were force-fed a dye marker, Sudan Red 7B, and confined with different proportions of unmarked termites. No significant mortality due to the dye was detected for up to 8 weeks after the treatment in either species. The marked/unmarked proportion did not affect the retention of the dye or mortality. Workers of *C. formosanus* retained visible dye for 6 weeks, while significant numbers (10-50%) of marked *R. flavipes* lost the dye within two weeks. Sudan Red 7B is suitable for estimating population size of *C. formosanus* with the multiple-marking method where the mark-recapture period extends to 6 weeks. The dye is not an adequate marker for *R. flavipes* when the mark-recapture period is $\geq$ 2 weeks.

Key Words: Mark-recapture, *Copototermes formosanus*, *Reticulitermes flavipes*, mark retention, Sudan Red 7B.

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INTRODUCTION

A marking material is required for studies of population dynamics of subterranean termites under field conditions. Of the nine dyes tested by Lai et al. (1983), an oil-soluble dye, Sudan Red 7B, was found to reside the longest in and cause the least mortality of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. This dye has been successfully used for estimating the population size of *C. formosanus* field colonies (Lai 1977).

Su et al. (1983) later found that, with *C. formosanus*, the amount of ingested Sudan Red 7B could be quantified using a spectrophotometer to correlate dye titer with exposure time to dyed food. No significant level of the dye was found to be transferred among nestmates by trophallaxis; therefore, the dye was used to study the foraging patterns of *C. formosanus* in field colonies (Su et al. 1984).

The application of the mark-recapture technique to estimate the population of field colonies of social insects is not without its pitfalls (Ayre 1962). Su et al. (1984) reported that *C. formosanus* foragers select feeding sites at random, thus both marked and unmarked individuals can be caught with equal probability after a reassimilation period. Only the foraging population of the colony, however, is estimated when the trapping systems described by Tamashiro et al. (1973) or by Su and Scheffrahn (1986) are used.

The marked proportions of field populations of *C. formosanus* have been small (ca. 0.1%) in past studies resulting in a large variance within estimates (Lai 1977;

Esenther 1980). Colony population estimates may be improved by using the weighted mean model in a multiple mark-release program (Begon 1979). This method requires that the mark remain for a period longer than the 2-4 weeks used in our previous studies. The effect of the marked/unmarked ratio on the retention time and mortality of marked individuals has not been examined. Moreover, the mark-recapture technique has not been attempted with native subterranean termites such as *Reticulitermes* species.

In this study, we determined the extended effect of Sudan Reb 7B on *C. formosanus* and *R. flavipes* (Kollar) when grouped in various proportions of marked and unmarked individuals.

MATERIALS AND METHODS

Both termie species were collected in coastal Broward County, Fla., using the method of Su and Scheffrahn (1986). Only the undifferentiated larvae of at least the third instar ('workers') were used in this study. Termites were tested within one week of collection. Our preliminary results with multiple colonies for both species indicated that colony differences in their responses to Sudan Red 7B was minimum, thus only results from single colony of each species are presented in this report. Mean worker weights of tested colonies, determined by weighing five groups of ten termites each, were 4.4 ± 0.1 and 1.8 ± 0.2 (mg $\pm$ SE) for *C. formosanus* and *R. flavipes*, respectively.

Approximately 600 termites were placed in a petri dish (9.0 cm diam by 1.5 cm high) containing two filter disks (Whatman No. 1, 9.0 cm diam). The disks were previously impregnated with acetone or an acetone solution of Sudan Red 7B to deliver final concentrations of 0 or 1% (wt./wt.) for *C. formosanus*, and 0 or 0.5% for *R. flavipes*. Preliminary studies showed an unacceptably high mortality (50-90%) of *R. flavipes* after 10 days of forced-feeding on Sudan Red 7B concentrations $> 0.5\%$. Ten units containing stained paper and 15 units containing acetone-treated paper were prepared for each species and stored at 29 ± 1 °C. After 10 days of force-feeding on the treated papers, 100 termites were combined to yield marked-to-unmarked proportions of 0, 10, 20, 30, 40, and 100%. The termites were transferred to screw-top jars (4.4 cm diam by 4.4 cm high) each containing an 8 cm^3 cube of spruce sapwood (*Picea* spp.) covered with 25 ml acetone-washed sand and 10 ml deionized water. Five soldiers of *C. formosanus* and two soldiers of *R. flavipes* were added to jars of their respective species to approximate the soldier proportions of the source colonies.

Twelve units were assembled for each of the prepared marked proportions for each species. A total of 144 experimental units were stored in an environmental chamber at 29 ± 1 °C, ca. 99% RH and near-constant darkness. Three units, selected at random from each of the marked proportion $\times$ species combination, were disassembled at bi-weekly intervals for 2 to 8 weeks. The numbers of marked and unmarked individuals were recorded.

Significant differences ($\alpha = 0.05$) between the resultant marked proportion and the initial marked proportion were detected using a chi-square test. Marked, unmarked, and total mortalities were log-transformed and subjected to the analysis of variance (SAS 1985). Because mortality was expressed as the percentage of the survivors among the initial number of the marked or unmarked termites, survivors that lost the dye marker inflated marked-group mortalities. Similarly,

'negative' mortalities (> original numbers) were recorded for some unmarked groups. The total mortalities reflected the actual termite deaths. 'Negative' mortalities were excluded from the analyses.

RESULTS AND DISCUSSION

Regardless of the original marked proportion, all groups of *C. formosanus* retained the same marked/unmarked ratio up to six weeks after being stained (Table 1). A ca. 5% reduction of marked termites, due to loss of dye, was observed after 6-8 weeks in groups containing 100% marked *C. formosanus*. Most of the marked *C. formosanus* retained visible coloration eight weeks after staining. Lai et al. (1983) reported that when force-fed with 1.5% Sudan Red 7B, *C. formosanus* retained the marker for as long as 30 days. Our results showed that with a concentration as low as 1%, the marker was recognizable for at least six weeks.

Table 1. Percentage (± SE) of marked termites 2-8 weeks after being confined with 0-100% of marked individuals.

		Initial marked proportion (%)					
Spp.†	Time (weeks)	0	10	20	30	40	100
C	2	0	9.9 ± 0.8	20.2 ± 1.0	29.8 ± 1.3	39.7 ± 1.1	100
	4	0	10.1 ± 0.2	18.8 ± 1.0	27.9 ± 0.8	38.9 ± 0.4	100
	6	0	8.6 ± 0.9	20.1 ± 1.3	29.2 ± 1.4	38.0 ± 1.5	100
	8	0	10.4 ± 0.5	17.9 ± 0.5	27.7 ± 1.3	38.6 ± 1.3	95.0 ± 1.4*
R	2	0	5.0 ± 1.5*	12.2 ± 1.4*	19.9 ± 2.0*	26.3 ± 3.1*	89.1 ± 2.4*
	4	0	4.2 ± 0.3*	11.1 ± 2.0*	19.6 ± 2.2*	28.1 ± 2.0*	84.2 ± 2.6*
	6	0	3.6 ± 1.2*	9.6 ± 0.8*	14.1 ± 2.6*	24.7 ± 0.5*	70.8 ± 5.2*
	8	0	2.9 ± 0.8*	9.6 ± 2.0*	11.7 ± 1.7*	30.5 ± 16.0*	20.3 ± 9.1*

* indicates proportion of marked individuals significantly deviated from the initial proportion according to chi-square test (α = 0.05).
† C: *C. formosanus*, R: *R. flavipes*.

Retention of Sudan Red 7B in *C. formosanus* groups was not affected by the marked/unmarked ratio. The results suggest that *C. formosanus* workers will retain the dye marker for six weeks after release into the field colonies. Our experience with *C. formosanus* suggests that the marked termites are homogeneously distributed into field populations one week after release (Su et al. 1984). Adding the ten days required for the staining process, a 41 day period is needed to complete three cycles of sequential release. Our finding that Sudan Red 7B is recognized in *C. formosanus* for 42 days indicates that this dye satisfies the criteria for a multiple marking procedure with a weighted mean model for this species.

The marked proportion did not affect the bi-weekly mortalities for *C. formosanus*, including total, marked, or unmarked mortalities (Table 2). The overall total mortality at week eight was similar to the control mortality, i.e. ca. 20%. The marked mortality was generally higher than the unmarked mortality, indicating that some marked individuals lost the marker. This percentage, however, was low and did not significantly affect the resultant marked proportion of *C. formosanus* (Table 1).

Table 2. Total mortality (T), marked mortality (M), and unmarked mortality (U) of *C. formosanus* 2-8 weeks after being confined with 0-100% of marked individuals.*

	Time (weeks)	Initial marked proportion (%) 0	10	20	30	40	100
T	2	10.7 ± 1.8	12.3 ± 0.7	9.3 ± 0.3	8.3 ± 0.9	12.7 ± 1.9	13.3 ± 4.4
	4	15.7 ± 0.7	14.3 ± 4.1	11.3 ± 0.7	20.0 ± 3.5	17.0 ± 1.5	12.0 ± 3.8
	6	17.0 ± 2.5	18.0 ± 2.1	20.3 ± 3.9	19.3 ± 4.8	25.3 ± 7.5	29.0 ± 4.0
	8	20.7 ± 2.4	26.0 ± 0.6	19.7 ± 3.2	24.0 ± 1.5	26.7 ± 1.5	19.7 ± 1.7
M	2	—	13.3 ± 6.7	8.3 ± 4.4	8.9 ± 4.0	13.3 ± 2.2	13.3 ± 4.4
	4	—	13.3 ± 3.3	16.7 ± 4.4	25.6 ± 4.4	19.2 ± 2.2	12.0 ± 3.8
	6	—	30.0 ± 5.8	20.0 ± 5.0	21.1 ± 8.0	29.2 ± 6.8	29.0 ± 4.0
	8	—	23.3 ± 3.3	28.3 ± 3.3	30.0 ± 1.9	29.2 ± 3.0	23.7 ± 2.0
U	2	10.7 ± 1.8	12.2 ± 1.3	9.6 ± 1.1	8.1 ± 2.1	12.2 ± 2.9	—
	4	15.7 ± 0.7	14.4 ± 4.2	10.0 ± 1.3	17.6 ± 3.4	15.6 ± 1.1	—
	6	17.0 ± 2.5	16.7 ± 2.8	20.4 ± 4.7	18.6 ± 3.8	22.8 ± 8.7	—
	8	20.7 ± 2.4	26.3 ± 1.0	17.5 ± 4.4	21.4 ± 2.9	25.0 ± 1.9	—

* Figures are means of three replicates (±SE). Means within the same row were not significantly different from each other according to the analysis of variance (α0.05).

Contrary to *C. formosanus*, significant numbers (10-50%) of marked *R. flavipes* lost the dye two weeks after test assembly (Table 1). By week eight, only 20% of the 300 initially stained termites were recognizable marked. The low retention rates may be due to the lower concentration (0.5%) of the dye used for *R. flavipes* compared to the 1% *C. formosanus* exposures. The 1% exposure would have killed unacceptably high percentages of *R. flavipes* workers, but the lower concentration is more rapidly excreted.

The marked proportion did not affect the total mortality of *R. flavipes* at any of the bi-weekly observations (Table 3). The mortalities of *R. flavipes* at week eight were higher than those of *C. formosanus*, but they are not significantly different from their own control mortality. The failure for *R. flavipes* to retain the Sudan Red 7B marker was attributed, in part, to high mortality among marked individuals. Because some marked individuals lost the dye, the numbers of unmarked termites increased, resulting in lower unmarked mortality (and 'negative' mortalities) compared to the total mortality (Table 3). The results indicate that Sudan Red 7B is not an adequate marking material for *R. flavipes* when the release-recapture interval is ≥ 2 weeks.

Sudan Red 7B was selected from a group of dyes screened against *C. formosanus* (Lai et al. 1983). A similar screening is needed before a marker superior to Sudan Red 7B can be found for *R. flavipes*. If Sudan Reb 7B is used for *R. flavipes*, a shorter release-recapture interval must be considered.

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Table 3. Total mortality (T), marked mortality (M), and unmarked mortality (U) of *R. flavipes* 2-8 weeks after being confined with 0-100% of marked individuals.*

	Time (weeks)	Initial marked proportion (%) 0	10	20	30	40	100
T	2	2.7 ± 1.2	3.0 ± 2.5	7.3 ± 1.5	8.3 ± 3.8	11.7 ± 3.4	12.7 ± 3.8
	4	13.3 ± 3.0	12.7 ± 4.1	10.7 ± 2.9	15.0 ± 0.6	10.0 ± 3.2	15.7 ± 1.2
	6	21.7 ± 4.7	16.7 ± 1.8	20.7 ± 3.7	20.3 ± 2.4	23.0 ± 3.8	10.7 ± 3.2
	8	32.7 ± 5.8	31.3 ± 3.5	32.3 ± 4.7	28.7 ± 2.2	44.3 ± 20.2	23.7 ± 2.7
M	2	—	50.0 ± 15.3	43.3 ± 7.3	38.9 ± 8.0	41.7 ± 7.9	22.0 ± 5.6
	4	—	63.3 ± 3.3	50.0 ± 10.0	44.4 ± 5.9	36.7 ± 5.8	29.0 ± 1.5
	6	—	70.0 ± 10.0	61.7 ± 4.4	62.2 ± 7.7	52.5 ± 1.4	37.0 ± 3.6
	8	—	80.0 ± 5.8	68.3 ± 4.4	72.2 ± 4.4	73.3 ± 1.7	84.3 ± 7.2
U	2	2.7 ± 1.2	(-2.2 ± 1.1)	(-1.7 ± 0.4)	(-4.8 ± 3.7)	(-8.3 ± 4.2)	—
	4	13.3 ± 3.0	7.0 ± 4.3	0.8 ± 1.7	2.4 ± 3.6	(-7.8 ± 3.6)	—
	6	21.7 ± 4.7	10.7 ± 1.0	10.4 ± 3.7	2.4 ± 2.7	3.3 ± 5.4	—
	8	32.7 ± 5.8	25.9 ± 3.5	23.3 ± 6.7	10.0 ± 3.0	25.0 ± 32.9	—

* Figures are means of three replicates (± SE). Means within the same row were not significantly different from each other according to the analysis of variance ($\alpha = 0.05$). Figures in parentheses were not included in the analysis.

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