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A short-term dye for marking eastern subterranean termites (*Reticulitermes flavipes* Koll.) (Isoptera, Rhinotermitidae)

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Abstract

The dye Sudan Blue 35 was evaluated as a material for labelling eastern subterranean termites, *Reticulitermes flavipes*, in foraging and behavioural studies. Termite workers were fed filter paper containing dye concentrations of 0.5, 1, 2, 4, 6, and 8 % for periods of 5–15 days. Concentrations greater than 2 % deterred feeding, which resulted in less effective labelling, and elicited significant mortality. In contrast to previous results with the dye marker Sudan Red 7B, very few termites retained the dye 15 days after feeding, independent of the original dye concentration. 80 % of *R. flavipes* workers fed 1 % dye for 10 days were still visibly labelled 5 days after feeding, with this proportion decreasing to 13 % after 10 days. As an alternative to other dye markers, Sudan Blue 35 would be suitable for field studies with *R. flavipes* employing mark-release-recapture cycles of 5–6 days, or for labelling termites in laboratory studies of behavioural interactions or feeding preferences.

1 Introduction

Subterranean termite (Rhinotermitidae) colonies occupy extensive networks of underground galleries (KING and SPINK 1969). In the urban environment, these galleries extend beneath pavement and ornamental plantings (GRACE et al. 1989; SU and SCHEFFRAHN 1988a). As a result, termite colony demographics and foraging behaviour are difficult to study under natural conditions.

Mark-release-recapture methodology has been successfully applied to study the colony foraging territories and populations of *Coptotermes formosanus* Shiraki (LAI 1977; SU and SCHEFFRAHN 1988a) and *Reticulitermes flavipes* (Kollar) (ESENTHER 1980; GRACE et al. 1989; SU and SCHEFFRAHN 1988b). Termites collected in traps containing wood or corrugated paper (GRACE 1989; SU and SCHEFFRAHN 1986) are fed paper impregnated with the oil-soluble dye Sudan Red 7B to colour the insects, then returned to the field site and recaptured at successive intervals. Since the dye is not passed noticeably by trophallaxis (GRACE and ABDALLAY 1989; SU et al. 1983), foraging distances can be determined by the traps from which termites are recaptured, and forager populations estimated from the proportion of marked individuals in each sample (BARONI-URBANI et al. 1978; JACKSON 1939).

Exposure of *R. flavipes* workers to paper containing high concentrations of Sudan Red 7B or long feeding periods on lesser dye concentrations results in both feeding deterrence and unacceptably high termite mortality. With dosages of 2 % or less and a 3–5 day feeding period, Sudan Red 7B is retained by *R. flavipes* for approximately 15–20 days (GRACE and ABDALLAY 1989). Therefore, an alternative dye is needed for longer mark-release-recapture cycles (SU et al. 1988), or if multiple releases are to be attempted within a short period of time (GRACE et al. 1989). In addition, use of a second dye would permit simultaneous releases of differently coloured termites, either to examine foraging interactions between nearby colonies or to improve the precision of estimates of the foraging population of a single colony. This study reports an evaluation of the dye Sudan Blue 35 (synonymous

with Fat Blue 35) as an alternative marking material for the eastern subterranean termite, *R. flavipes*.

2 Materials and methods

Eastern subterranean termites, *R. flavipes*, were collected in traps placed in the soil at a site in Scarborough, Ontario (43° 44′ N, 79° 16′ W). The trap (GRACE 1989) consisted of a 15 cm length of 10 cm diameter plastic pipe, capped at one end and buried vertically just below the soil surface. Two 4 cm diameter pipes containing rolled corrugated paper were placed within the larger pipe. The inner pipes, containing feeding termites, could then be removed and replaced without disturbing the permanent trap installation. Termites collected in the corrugated paper were kept in plastic boxes in an unlighted cabinet at 27 ± 0.5 °C and 90 ± 5 % relative humidity. Bioassays were also performed in this cabinet.

Acetone solutions of Fat (Sudan) Blue 35 dye (Sigma Chemical Company, St. Louis, Missouri) were applied evenly by pipet to each side of Whatman No. 1 filter papers to achieve precise dye concentrations (weight/weight percentage). The dyed filter paper strips, each ca 2 × 6 cm and 69 mg, were oven-dried and weighed at a precision of 0.01 mg before and after exposure to termites to evaluate feeding. In each bioassay, a single paper strip was placed along one side of a 44.8 ml polystyrene vial (60 × 36 mm diameter) containing 7 ml (10 g) oven-dried white sand, 4 ml perlite, and 2 ml de-ionized water. An additional 1 ml of water was added at 10 day intervals to maintain soil moisture. *Reticulitermes flavipes* workers, older than the third instar, were deposited in the vial, which was then capped with a polyurethane foam plug and placed in the incubator.

In the first experiment, *R. flavipes* workers (40 workers per vial, 4 vials per treatment) were fed papers containing dye concentrations of 0 (control), 0.5, 1, and 2 % for either 5 or 15 days. These concentrations were comparable to those previously determined to be suitable for marking *R. flavipes* with Sudan Red 7B (GRACE and ABDALLAY 1989). To evaluate post-exposure mortality, feeding, and dye retention, 2 groups of 25 of the workers exposed to each dye concentration for 15 days were placed in vials containing clean filter paper strips and maintained under the same experimental conditions for an additional 15 or 30 days.

A second experiment was conducted to determine whether feeding on higher concentrations of Sudan Blue 35 would improve dye retention. Under the same conditions as the first experiment, 4 groups of 40 termite workers per treatment were fed papers impregnated with 4, 6, and 8 % dye for a 10 day period. Following this exposure period, 2 groups of 14–20 dyed workers from each treatment were placed in similar vials with clean filter papers and dye retention evaluated after an additional 15 days.

A third experiment was performed to measure the decay rate of the dye in termites fed a 1 % concentration for 10 days, identified as a suitable target concentration and exposure period in the preceding work. Following the 10 day exposure, 2 groups of 25 dyed workers, confined to vials containing clean filter papers under the same experimental conditions, were evaluated each day (24 hours) over the following 10 days.

Mortality, percentages of the survivors showing visible coloration, and paper weight loss data from each experiment were subjected to analysis of variance with a one-way layout, and treatment means significantly different at the 0.05 level were separated by the Ryan-Einot-Gabriel-Welsch multiple F test (SAS Institute 1987).

3 Results

Exposure to 0.5, 1, or 2 % Sudan Blue 35 dye for 5 days effectively coloured *R. flavipes* workers without inducing mortality or providing any systematic evidence of feeding deterrence (table 1). However, when the exposure period was extended to 15 days, significantly greater mortality and a concomitant decrease in feeding was noted in the workers exposed to 2 % dye. No evidence of delayed mortality or feeding suppression was observed as a consequence of any of these treatments either 15 or 30 days after the 15 day dye exposure (table 2).

Although *R. flavipes* workers were rapidly coloured by feeding on 0.5, 1. or 2 % dye (table 1), very few retained visible coloration 15 days after exposure (table 2). Exposure to greater dye concentrations of 4, 6, or 8 % did not increase dye longevity (table 3). Moreover, feeding was reduced on papers treated with these high concentrations and mortality was unacceptably high. Concentration-dependent feeding deterrence was evi-

Table 1. **Mean (± SD) percent mortality, paper consumption (feeding), and percent of surviving *R. flavipes* workers coloured after feeding on papers treated with Sudan Blue 35 dye[1]**

Dye concentration %	5 Day dye exposure			15 Day dye exposure		
	Mortality %	Coloured %	Feeding mg	Mortality %	Coloured %	Feeding mg
0	4 ± 3a	–	16.9 ± 3.0a	6 ± 4a	–	35.8 ± 3.0ab
0.5	4 ± 1a	99 ± 1a	5.7 ± 3.2b	13 ± 3ab	99 ± 1a	40.4 ± 9.9a
1.0	4 ± 4a	100 ± 0a	10.5 ± 4.6ab	14 ± 7ab	100 ± 0a	28.0 ± 5.3bc
2.0	4 ± 1a	100 ± 0a	9.7 ± 7.4ab	20 ± 6b	99 ± 1a	17.5 ± 4.3c

[1] Each mean represents four groups of 40 workers. Means in each column followed by the same letter are not significantly different at the 0.05 level.

Table 2. **Mean (± SD) percent mortality, paper consumption, and percent of surviving *R. flavipes* workers coloured 15 and 30 days after feeding on papers treated with Sudan Blue 35 dye for 15 days[1]**

Dye concentration %	15 Days after dye exposure			30 Days after dye exposure		
	Mortality %	Coloured %	Feeding mg	Mortality %	Coloured %	Feeding mg
0	20 ± 11	–	6.1 ± 2.6	34 ± 3	–	19.0 ± 4.1
0.5	18 ± 3	10 ± 7	7.1 ± 0.1	24 ± 6	6 ± 8	23.8 ± 4.2
1.0	22 ± 8	10 ± 1	4.5 ± 0.7	28 ± 0	8 ± 4	30.8 ± 1.2
2.0	14 ± 3	19 ± 6	5.8 ± 5.2	48 ± 11	12 ± 9	15.6 ± 5.0

[1] Each mean represents 2 groups of 25 workers. There were no significant differences among means within each column at the 0.05 level.

Table 3. **Mean (± SD) percent mortality, paper consumption, and percent of surviving *R. flavipes* workers coloured after feeding for 10 days on high concentrations of Sudan Blue 35 dye, and the percent of surviving termites still coloured 15 days after dye exposure**

Dye Concentration %	Mortality[1] %	Coloured[1] %	Feeding[1] %	Percent retaining dye for 15 days[2]
4	0 ± 0a	73 ± 10a	23 ± 9a	0 ± 0a
6	18 ± 9b	65 ± 6a	36 ± 29a	11 ± 16a
8	11 ± 11ab	74 ± 6a	43 ± 7a	11 ± 7a

[1] Each mean represents 4 groups of 40 workers, with mortality and feeding expressed as a percentage of the control by ABBOTT's (1925) formula – [2] Each mean represents 2 groups of 14–20 workers. Means in each column followed by the same letter are not significantly different at the 0.05 level.

denced by a 25–35 % decrease in the number of individuals visibly coloured after exposure to the 4, 6, and 8 % dye-treated papers.

Most termites fed 1 % dye for 10 days (table 4) retained the coloration for 5–6 days. The proportion of coloured individuals then rapidly declined to 13 % by the tenth day after dye exposure.

4 Discussion

Use of the dye Sudan Red 7B in mark-release-recapture studies has provided valuable information on the behavioural ecology of *C. formosanus* (SU and SCHEFFRAHN 1988a) and

Table 4. **Mean (± SD) number of *R. flavipes* workers retaining coloration after feeding on papers treated with 1 % Sudan Blue 35 dye for 10 days[1]**

Days after dye exposure									
1	2	3	4	5	6	7	8	9	10
100a ± 0	71ab ± 12	82ab ± 2	83ab ± 13	80ab ± 6	66ab ± 1	53bc ± 11	31cd ± 14	31cd ± 7	13d ± 0

[1] Two groups of 25 workers were evaluted each day. Means followed by the same letter are not significantly different at the 0.05 level.

R. flavipes (Grace et al. 1989). Methods such as excavation of subterranean termite colonies (Howard et al. 1982; King and Spink 1969) are impractical in urban locations, where these species are serious structural pests. To improve the precision of *C. formosanus* forager population estimates, Su and Scheffrahn (1988a) employed multiple release-recapture cycles. Alternatively, Grace et al. (1989) derived their estimates from multiple recaptures following a single release of marked individuals, in order to accommodate the short ca. 4 month period of peak *R. flavipes* foraging activity in Toronto.

Our results indicate that Sudan Blue 35 dye is not an equivalent replacement for Sudan Red 7B in field studies. At concentrations of 2 % or less, neither dye induces significant mortality nor deters feeding. However, Sudan Blue 35 is much more rapidly excreted, lasting 5–6 days in contrast to the 15–20 day dye retention by *R. flavipes* after feeding on comparable levels of Sudan Red 7B (Grace and Abdallay 1989). It is possible that Sudan Blue 35 might persist longer in *C. formosanus* than in *R. flavipes*, as does Sudan Red 7B (Su et al. 1988).

The short retention time of Sudan Blue 35 indicates that it is suitable for use in mark-release-recapture cycles with *R. flavipes* lasting less than 6 days. There would appear to be no advantage to the use of dye concentrations greater than 1 % or feeding periods exceeding 10 days. This dye would also be appropriate for labelling specific groups or individual termites in laboratory assays of feeding preference or intercolony agonistic interactions.

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Zusammenfassung

Ein Farbstoff für kurzzeitige Markierung der Termitenart Reticulitermes flavipes Koll.

Der Farbstoff Sudan Blau 35 wurde zur Markierung der Termitenart *Reticulitermes flavipes* in Studien über Verhalten und Nahrungssuche gebraucht. Arbeiter der Termiten wurden 5 bis 15 Tage lang mit Filterpapier gefüttert, das eine Farbstoffkonzentration von 0.5, 1, 2, 4, 6, und 8 % enthielt. Farbstoffkonzentrationen größer als 2 % störten die Futteraufnahme, was eine geringere Markierung und signifikante Mortalität zur Folge hatte. Im Gegensatz zu vorhergehenden Ergebnissen mit dem Farbstoff Sudan Rot 7B und unabhängig von der Ausgangskonzentration, behielten nur wenige Termiten den Farbstoff länger als 15 Tage nach der Fütterung. Fünf Tage nach der Fütterung waren 80 % der *R. flavipes* Arbeiter, die vorher 10 Tage lang mit 1 % Farbstoff gefüttert worden, waren noch deutlich markiert. Dieser Prozentsatz verringerte sich auf 13 % 10 Tage nach der Fütterung. Als Alternative für andere Farbstoffe wäre Sudan Blau 35 für Feldversuche mit *R. flavipes* mit Versuchszyklen zwischen 5 und 6 Tagen geeignet, ebenso zur Markierung von Termiten für Verhaltensstudien im Labor und für Studien über bevorzugte Nahrungsaufnahme.

References

SAS INSTITUTE INC., 1987: SAS/STAT Guide for Personal Computers, Version 6 Ed. Cary, NC: SAS Institute Inc.

BARONI-URBANI, C.; JONES, G.; PEAKIN, G. J., 1978: Empirical data and demographic parameters. In: Production Ecology of Ants and Termites. Ed. by BRIAN, M. V., New York: Cambridge Univ. Press. pp. 5–44.

ESENTHER, G. R., 1980: Estimating the size of subterranean termite colonies by a release-recapture technique. Internat. Research Group on Wood Preserv. Doc. No. IRG/WP/112.

GRACE, J. K., 1989: A modified trap technique for monitoring *Reticulitermes* subterranean termite populations (Isoptera: Rhinotermitidae). Pan-Pac. Entomol. (in press).

GRACE, J. K.; ABDALLAY, A., 1989: Evaluation of the dye marker Sudan Red 7B with *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Sociobiology **15**, 71–77.

GRACE, J. K.; ABDALLAY, A.; FARR, K. R., 1989: Eastern subterranean termite (Isoptera: Rhinotermitidae) foraging territories and populations in Toronto. Can. Ent. **121**, 551–556.

HOWARD, R. W.; JONES, S. C.; MAULDIN, J. K.; BEAL, R. H., 1982: Abundance, distribution, and colony size estimates for *Reticulitermes* spp. (Isoptera: Rhinotermitidae) in southern Mississippi. Environ. Entomol. **11**, 1290–1293.

JACKSON, C. H. N., 1939: The analysis of an animal population. J. Anim. Ecol. **8**, 238–246.

KING, E. G. JR.; SPINK, W. T., 1969: Foraging galleries of the Formosan subterranean termite, *Coptotermes formosanus,* in Louisiana. Ann. Entomol. Soc. Am. **62**, 536–542.

LAI, P.-Y., 1977: Biology and ecology of the Formosan subterranean termite, *Coptotermes formosanus,* and its susceptibility to the entomogenous fungi, *Beauveria bassiana* and *Metarrhizium anisopliae*. Ph. D. Diss., Univ. of Hawaii, Honolulu.

SU, N.-Y.; SCHEFFRAHN, R. H., 1986: A method to access, trap, and monitor field populations of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in the urban environment. Sociobiology **12**, 299–304.

— — 1988a: Foraging population and territory of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in an urban environment. Sociobiology **14**, 353–359.

— — 1988b: Intra- and interspecific competition of the Formosan and the eastern subterranean termite: evidence from field observations (Isoptera: Rhinotermitidae). Sociobiology **14**, 157–164.

SU, N.-Y.; SCHEFFRAHN, R. H.; BAN, P., 1988: Retention time and toxicity of a dye marker, Sudan Red 7B, on Formosan and eastern subterranean termites (Isoptera: Rhinotermitidae). J. Entomol. Sci. **23**, 235–239.

SU, N.-Y.; TAMASHIRO, M.; YATES, J.; LAI, P.-Y.; HAVERTY, M. I., 1983: A dye, Sudan Red 7B, as a marking material for foraging studies with the Formosan subterranean termite. Sociobiology **8**, 91–97.

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