Evaluation of Twelve Dye Markers for Population Studies of the Eastern and Formosan Subterranean Termite (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Twelve biological stains were evaluated for their suitability as dye markers for population studies of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, and the eastern subterranean termite, *Reticulitermes flavipes* (Kollar). At the proper concentrations and exposure time, Nile Blue A and Neutral Red remained visible in both termite species throughout the 15-day period and did not cause significant mortality. Sudan Red 7B and Sudan Black B were well retained in *C. formosanus* with little toxicity, but caused significant mortality of *R. flavipes*. No significant toxicity was exhibited by Sudan IV against both termites species. *R. flavipes* retained Sudan IV marker well, but this dye was excreted rapidly from *C. formosanus*.

INTRODUCTION

A mark-release-recapture program is frequently used for investigation of animal populations (Southwood 1971). Due to intracolony polyethism, a mark-recapture method may only provide an estimate of the foraging population instead that of the entire colony of social insects (Ayre 1962). Despite this shortcoming, the mark-recapture method remains the only viable approach in many circumstances (Baroni-Urbani *et al.* 1978). It is a particularly useful tool for studies of the underground populations of subterranean termites.

The oil-soluble dye, Sudan Red 7B, has been used to estimate foraging populations and to study the cryptic foraging territories

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of subterranean termites (Lai 1977, Lai *et al.* 1983, Su & Scheffrahn 1988, Grace 1990, Jones 1990). This dye marker was also instrumental in the evaluation of a bait-toxicant application against field colonies of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki (Su *et al.*, in press). Sudan Red 7B, however, caused unacceptably high mortality of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), at high concentrations. This dye was successfully retained in *R. flavipes* less than two weeks when lower concentrations were used (Su *et al.* 1988). Grace (1989) confirmed our results that Sudan Red 7B had a short retention period in *R. flavipes* at non-toxic loads, but indicated that it could be used in a short-term mark-recapture program.

Recently, we employed a weighted mean model with a multiple capture-recapture procedure to improve the accuracy of the estimates of foraging populations of *C. formosanus* (Su & Scheffrahn 1988). The procedure requires a marker that exhibits minimum lethal effects against termites yet remains in termites long enough (ideally 4-8 weeks) to complete several (>3) capture-recapture cycles (Su *et al.* 1988).

Although Sudan Red 7B has been used for marking *C. formosanus* for over a decade, other mark-recapture models such as the triple catch method (Begon 1979) have not been possible because marking was limited to one color. The development of additional dye marker(s) with other color(s) would enhance the information that could be extracted from cryptic populations of the subterranean termites by using multiple marking programs.

To be used in a mark-recapture scheme, a dye marker must not cause substantial mortality (both immediate and delayed mortality) and visibly reside in dyed termites during the entire sampling period. Also, no detectable level of a dye should be transferred among nestmates through trophallaxis. This study was done to evaluate dye markers suitable for *C. formosanus* and *R. flavipes*.

MATERIALS AND METHODS

Twelve dyes were evaluated: Nile Blue A (96%), Sudan Black B (50%), Sudan Red 7B (95%) (Aldrich Chemical Company, Milwaukee, WI), Azure A (100%), Erythrothin B (85%), Fuchsin Acid (70%), Neutral Red (67%), Rhodamine B (93%), Rose Bengal (98%), Solvent Blue 35 (97%), Sudan III (91%), and Sudan IV (85%) (Sigma Chemical Company, St. Louis, MO). These compounds were selected from biological stains that are used for

dyeing animal tissues, lipids, or cell granules. Solvents used to dissolve the dyes included; water for Azure A, Fuchsin Acid, and Rose Bengal; ethanol for Erythrothin B, Neutral Red, and Rhodamine B; chloroform for Sudan III and Sudan IV; and acetone for Nile Blue A, Solvent Blue 35, Sudan Black B, and Sudan Red 7B. Dye solutions or solvents alone were evenly poured onto two filter papers (Whatman No. 1, 5.5cm) placed in a petri dish (5.5cm by 1.5cm high). Solvent evaporation overnight yielded dye concentrations of 0, 0.05, 0.25, and 0.5% (wt/wt). After the addition of 0.5ml of deionized water, approximately 100 termites each were introduced into the petri dish containing treated papers. Two staining units were prepared for each dye and stored at 28 ± 1°C. Worker termites (undifferentiated larvae of >3rd instar) were confined in the dishes and forced fed on treated papers for 3 or 6 days after which twenty stained workers were selected at random and transferred to a petri dish (5.5cm by 1.5cm high) containing two moistened filter papers (Whatman No. 1, 5.5cm). Five soldiers and one soldier were added to the stained worker groups for *C. formosanus* and *R. flavipes*, respectively. Observations were made daily and dead termites removed. Numbers of stained termites and survivors were recorded every three days up to 15 days. For each termite species, the test was repeated three times using termites collected from three colonies.

RESULTS AND DISCUSSION

Retention and toxicity of the dyes on termites are presented in Figs. 1-8. The results were divided into dye groups that were dissolved in water, ethanol, acetone, or chloroform.

Azure A, Acid Fuchsin and Rose Bengal. Results of these three dyes that were dissolved in water for impregnation of feeding filter papers are presented in Fig. 1 and 2 for the Formosan subterranean termite and eastern subterranean termite, respectively. At the lowest concentration (0.05%) and 3-day exposure, Azure A did not cause significant mortality for either termite species, but termites were not sufficiently stained. When the exposure time and/or concentrations were increased, more termites retained the Azure A marker, but mortality became unacceptably high.

Acid Fuchsin caused little mortality of either termite species

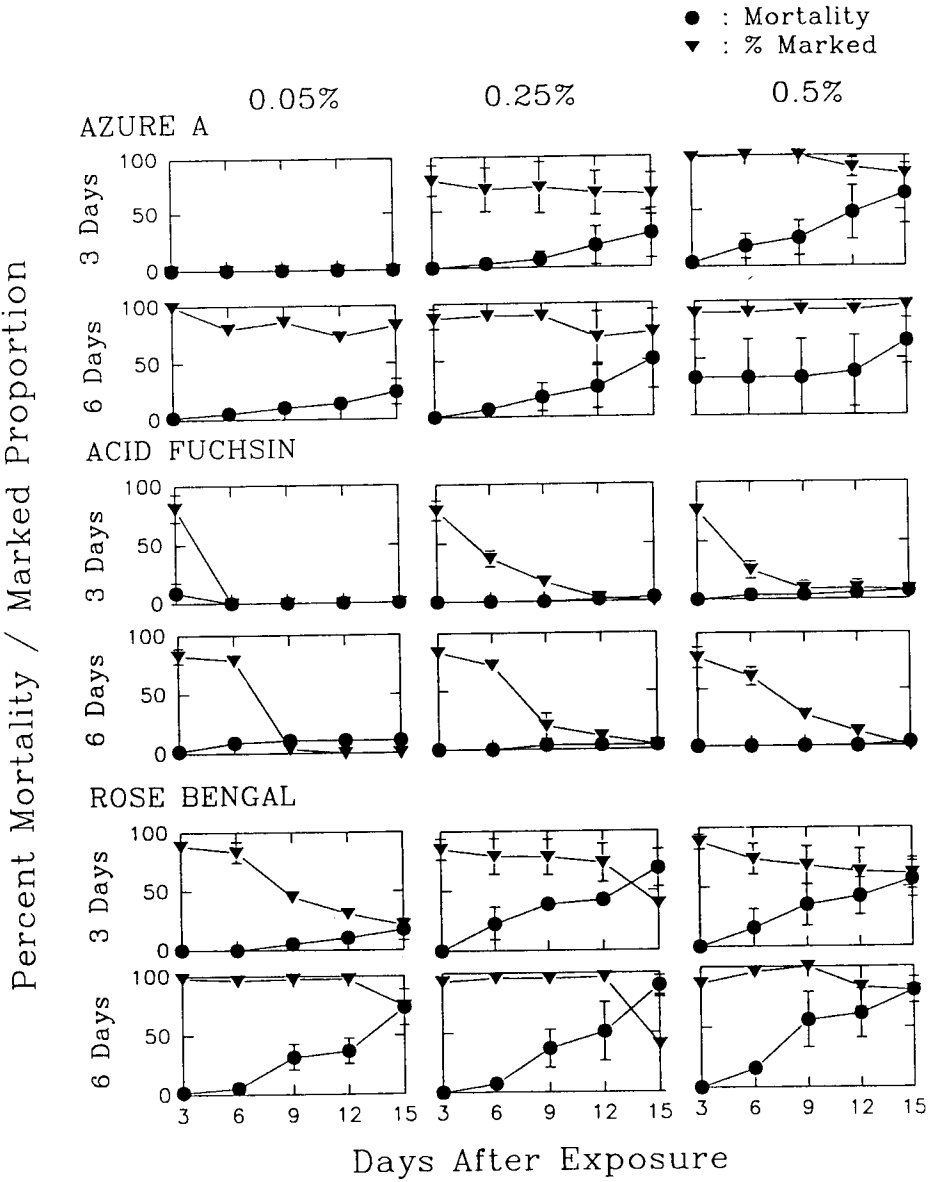

Fig 1. Retention and toxicity of three dyes, Azure A, Acid Fuchsin and Rose Bengal against *C. formosanus*.

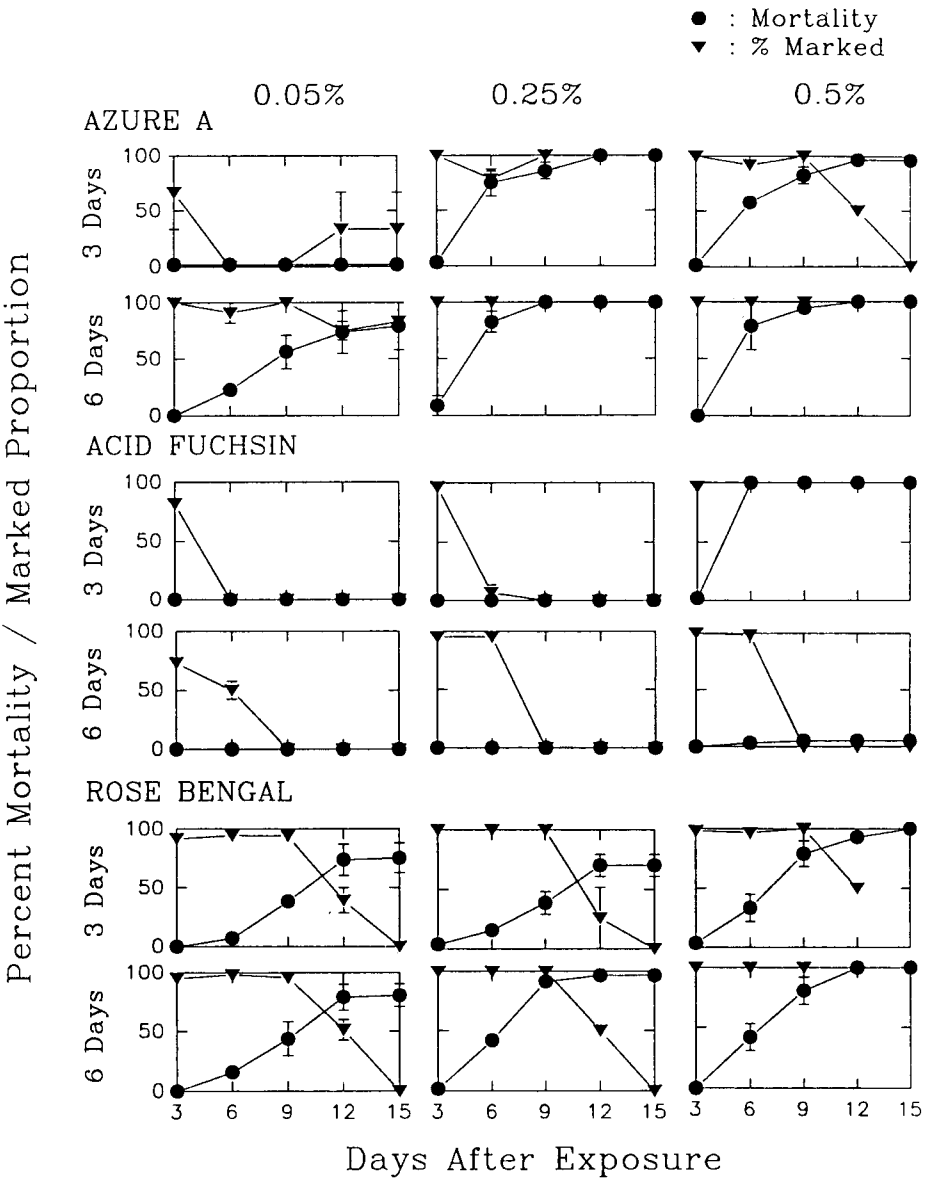

Fig 2. Retention and toxicity of three dyes, Azure A, Acid Fuchsin and Rose Bengal against *R. flavipes*.

(except for *R. flavipes* exposed to 0.5% for 3 days), but its stain vanished within a week after the exposure. Regardless of the concentration or exposure time, retention of Rose Bengal decreased while its delayed toxicity become evident against both *C. formosanus* and *R. flavipes*. The results indicated that none of these three dyes can be used for either termite species.

Erythrosin B, Neutral Red and Rhodamine B. Results of these dyes that were dissolved in ethanol for testing are presented in Fig. 3 and 4. Erythrosin B was not highly toxic, but this dye was rapidly excreted from termites.

Neutral Red caused little mortality of *C. formosanus* at all tested concentrations, yet its marking remained visible 15 days after the exposure. Although significant delayed mortality was recorded from *R. flavipes* exposed to 0.25% Neutral Red for 6 days or 0.5%, low mortality and high retention rates were observed from groups exposed to Neutral Red at 0.25% for 3 days or 0.05% (Fig. 4). Except for 0.5% and 6 days exposure that caused high mortality at 15 days, Rhodamine B marking did not persist in *C. formosanus*. Similarly, the high retention rate of Rhodamine B recorded from *R. flavipes* corresponded with high mortality. Of the three dyes in this group, therefore, Neutral Red may be useful as a marker for both termite species.

Nile Blue A, Sudan Red 7B, Sudan Black B and Solvent Blue 35. Acetone solutions were used to stain the feeding papers for these four dyes. Retention of these dyes in termites and associated mortalities are illustrated in Fig. 5 and 6. Nile Blue A caused little mortality and remained visible in the Formosan subterranean termite throughout the entire experiment. Substantial numbers of *R. flavipes* fed on papers containing 0.5% (for 3 or 6 days) or 0.25% Nile Blue A for 6 days were killed one week after the exposure. Coloration from Nile Blue A was retained in *R. flavipes* with low mortality when termites were fed with concentrations of 0.05 or 0.25% for 3 days.

Sudan Red 7B, a widely used dye for marking subterranean termites, exhibited low toxicity against *C. formosanus* and remained visible in this termite 15 days after the exposure when concentrations of 0.25 or 0.5% were used. For *R. flavipes*, however, Sudan Red 7B was either too toxic (at 0.25 or 0.5%), or did not visibly persist at a concentration of 0.05%. This confirmed our previous report that Sudan Red 7B is not an adequate maker for *R. flavipes*

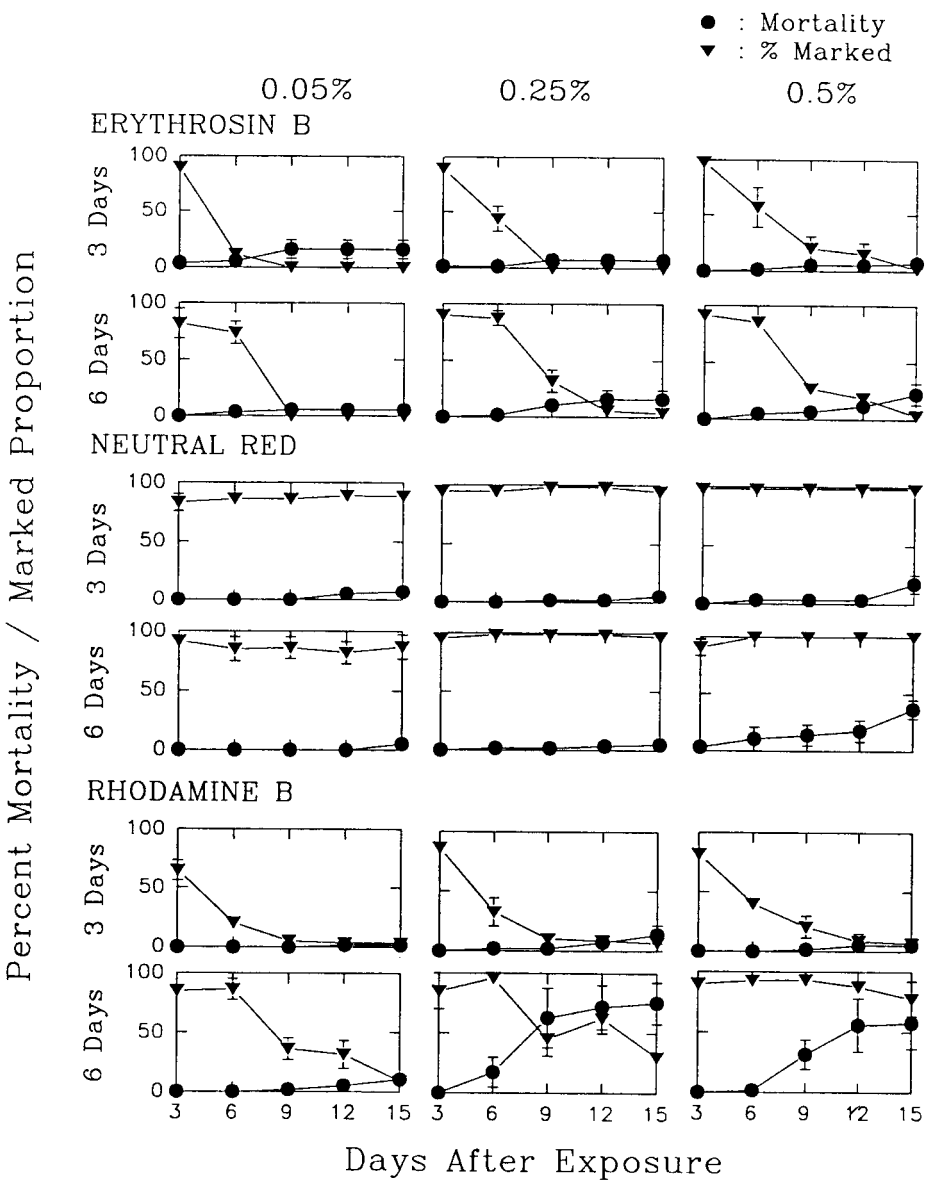

Fig 3. Retention and toxicity of three dyes, Erythrosin B, Neutral Red and Rhodamine B, against *C. formosanus*.

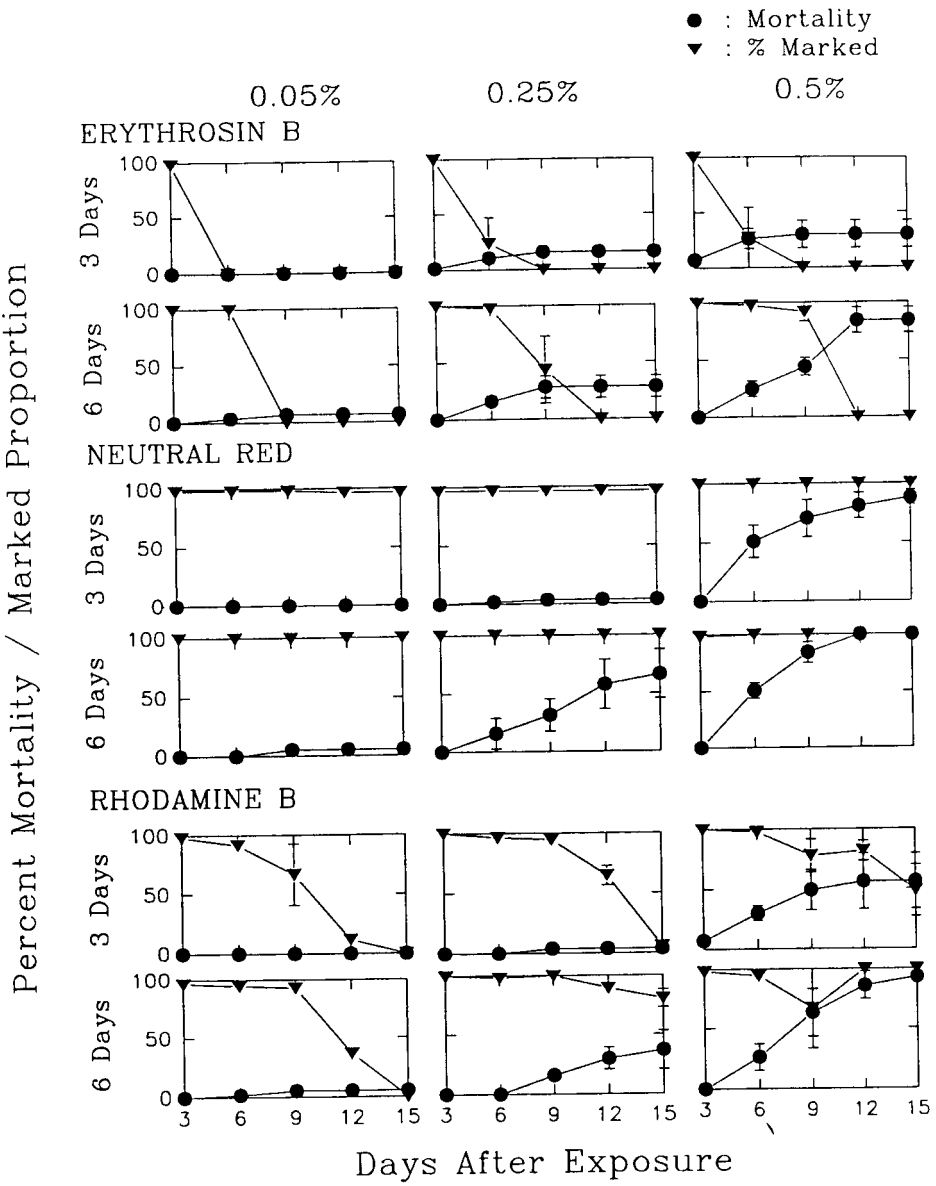

Fig 4. Retention and toxicity of three dyes, Erythrosin B, Neutral Red and Rhodamine B, against *R. flavipes*.

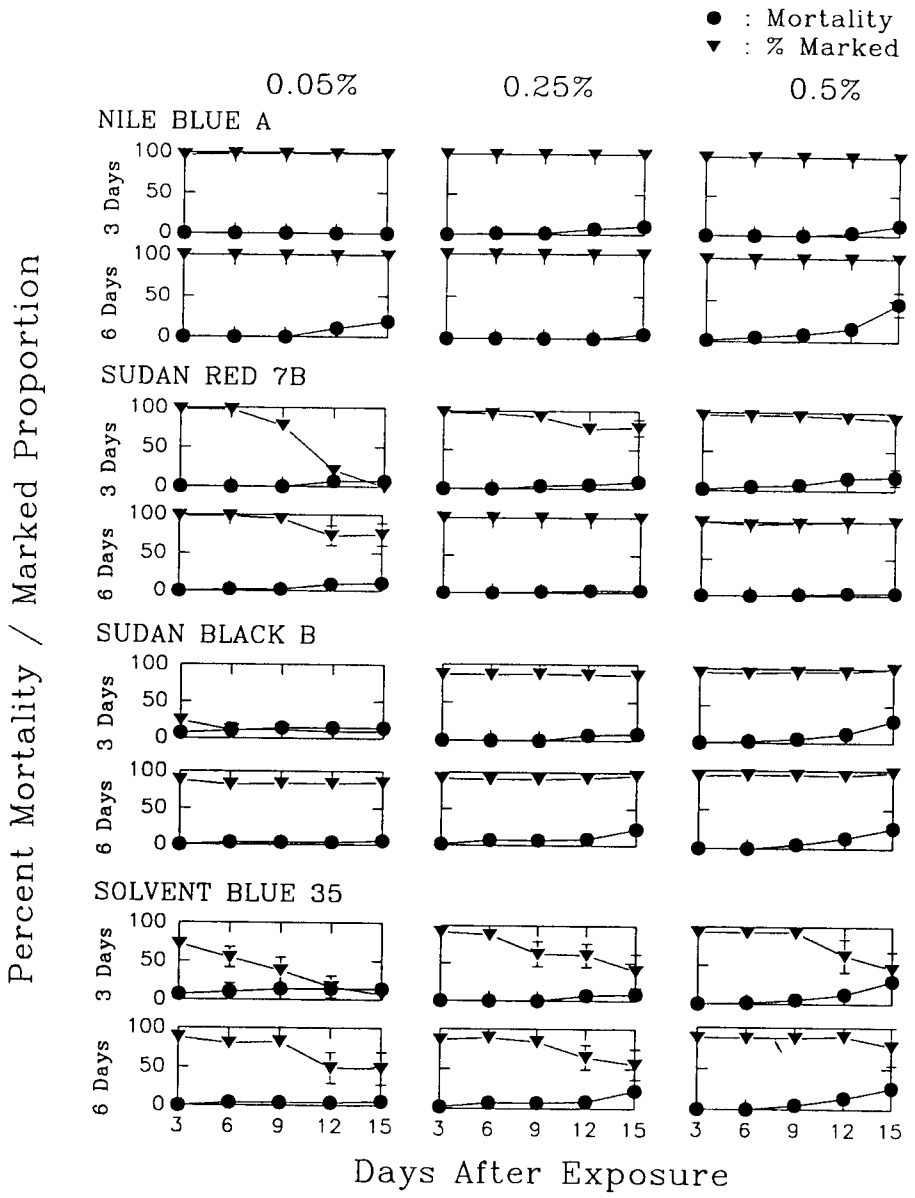

Fig 5. Retention and toxicity of four dyes, Nile Blue A, Sudan Red 7B, Sudan Black B and Solvent Blue 35 against *C. formosanus*.

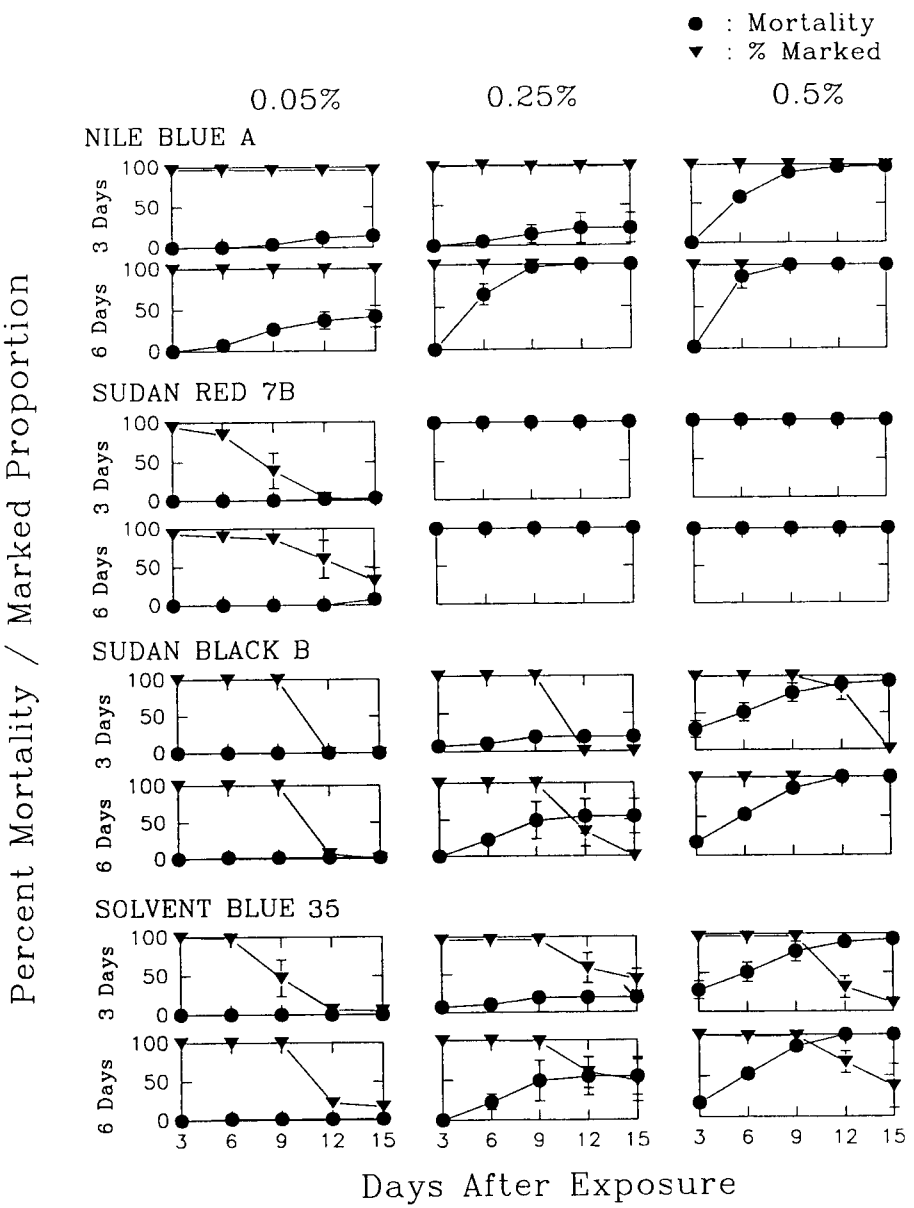

Fig 6. Retention and toxicity of four dyes, Nile Blue A, Sudan Red 7B, Sudan Black B and Solvent Blue 35 against *R. flavipes*.

(Su *et al.* 1988).

Except for those exposed to a concentration of 0.05% for 3 days, Sudan Black B remained visible in *C. formosanus* at the end of the 15-day period. Only at the concentration of 0.5% of Sudan Black B were significant mortalities observed from *C. formosanus*. On the contrary, the majority of *R. flavipes* exposed to Sudan Black B either did not retain their coloration or died. For both termite species, Solvent Blue 35 generally was not visibly retained at lower concentrations, or caused substantial mortality at higher concentrations.

Of these four dyes tested, Nile Blue A appeared to be the most useful dye marker for both termite species. In addition to Sudan Red 7B, Sudan Black B may also be used for *C. formosanus*.

Sudan III and Sudan IV. Chloroform was used to prepare the solution for these two dyes. Neither dye was retained in *C. formosanus* (Fig. 7). Sudan III vanished rapidly in *R. flavipes* except for those fed on 0.5% for 6 days; this group, however, suffered from high mortality (Fig. 8). *R. flavipes* fed on papers stained with 0.25% for 6 days, or 0.5% for 3 days, generally maintained the marking throughout the test period with minimal mortality. Of these two dyes, Sudan IV may be a useful marker for *R. flavipes*.

Although the tested dyes were developed for staining animal tissue, they are generally toxic against termites (Delaplane *et al.* 1988). Of the 12 dyes screened, Nile Blue A and Neutral Red (at proper concentrations and exposure time) remained visible in both termite species for 15 days period and did not cause significant mortality. Sudan Red 7B and Sudan Black B were retained in *C. formosanus* with little toxicity, but caused significant mortality of *R. flavipes*. With adequate concentrations and exposure time, Sudan IV may be a useful dye marker for *R. flavipes*, but it was excreted rapidly from *C. formosanus*. Although these dyes are promising, additional studies are needed to determine the long term retention and possible delayed toxicity (30-40 days), and to examine the potential for and extent of trophallactic transfers among nestmates.

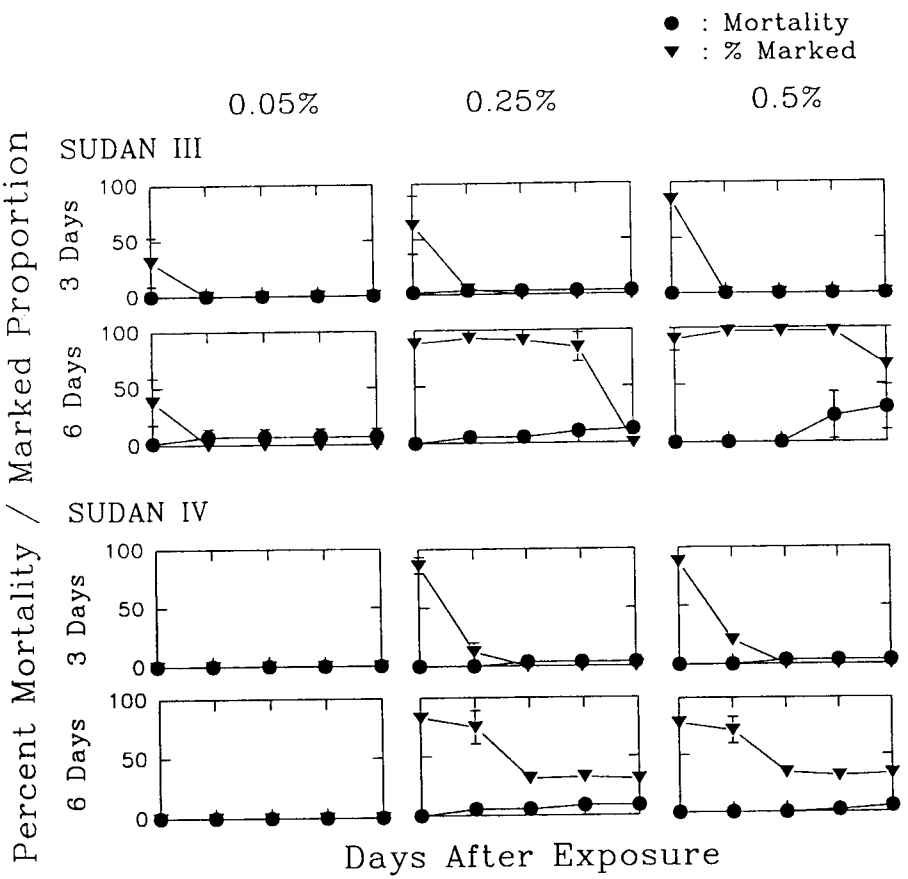

Fig 7. Retention and toxicity of two dyes, Sudan III and Sudan IV against *C. formosanus*.

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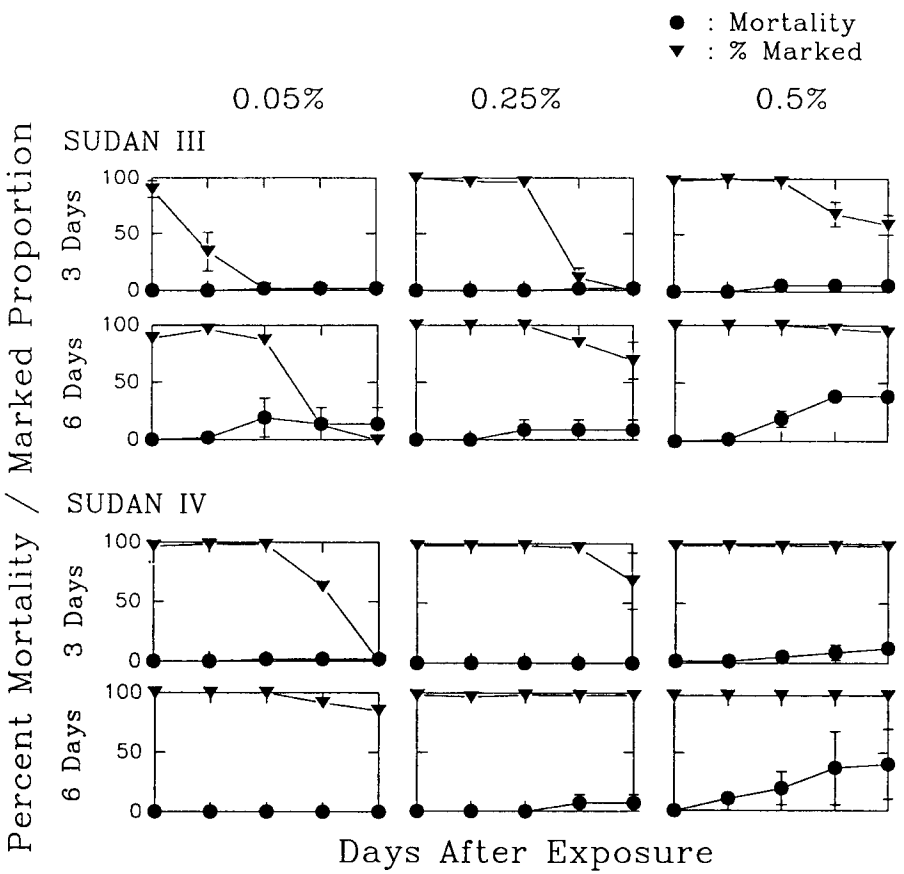

Fig 8. Retention and toxicity of two dyes, Sudan III and Sudan IV against *R. flavipes*.

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