PURPLE DYE-MARKER FOR *RETICULITERMES* SPP. (ISOPTERA: RHINOTERMITIDAE)

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Of the many dye markers evaluated for subterranean termites, only Neutral Red and Nile Blue A have been shown to be suitable persistent stains (>7 d) (Su et al. 1991, Oi and Su 1994, Evans 1997). The scarcity of persistent dye markers available has limited field studies for examining the interactions of multiple subterranean termite colonies using mark-recapture methods. Purple is a color that can be seen easily in the cream colored subterranean termite workers. However, several violet and purple stains have been shown to be toxic (Oi and Su 1994).

In order to create the purple marker, 0.5% (wt/wt) Neutral Red (59% dye content, N-129, Lot 974465A, Fisher, Fair Lawn, NJ) and 0.1% (wt/wt) Nile Blue A (87% dye content, Aldrich 12,147-9 #12, Lot L200916H2) were mixed separately as solutions. The solutions then were combined in proportions of 75:25, 50:50, and 25:75 (Neutral Red:Nile Blue A) to produce different shades of purple. Thus, to make a shade of purple in a 75:25 proportion, 75 ml of 0.5% (wt/wt) Neutral Red solution was combined with 25 ml of 0.1% (wt/wt) Nile Blue A solution.

Filter papers (9 cm, Whatman 2) stained with 0.5% (wt/wt) Neutral Red only or with 0.1% (wt/wt) Nile Blue A only were used as the standard control stains. All stains were applied at a rate of 1.5 ml per filter paper. Unstained filter paper was used as an untreated control.

Stained filter paper disks were allowed to air dry for at least 24 h and then placed in plastic Petri dishes (100 diam., 15 mm high). One ml of distilled water was added to the filter paper, then 200 worker termites were placed in each dish and allowed to feed for 5 d. *Reticulitermes virginicus* workers from two colonies were collected in the Austin Cary Forest, Waldo, FL, and the Smathers Library West, University of Florida, Gainesville, FL. Two replicates of each stain combination and controls were used. The experiment was designed as a randomized complete block, blocking on colony.

Percent survival was recorded after 5 d. Data were analyzed using analysis of variance (SAS Institute 1998). Stains did not cause significant mortality at the end of the 5 d feeding period ($F = 0.66$, $P = 0.6670$, $df = 5,11$) (Table 1).

Since there was no significant mortality after feeding on the stained filter paper for 5 d, two sets of 20 termites from each experimental unit were placed in another Petri dish (60 × 15 mm) to evaluate delayed mortality. The Petri dish was lined with unstained filter paper (5.5 cm, Whatman 1) that was moistened with about 0.5 ml distilled water. Standard control stains Neutral Red and Nile Blue A were used to visually compare the staining intensity of the purple stains over a 14 d period.

Percent survival was recorded after 14 days and data were analyzed using analysis of variance (SAS Institute 1998). Stains did not cause significant delayed mortality at the end of the 14 d feeding period ($F = 0.60$, $P = 0.7034$, $df = 5,22$) (Table 1). Stains were still visible in all termite workers at the end of 14 d in filter paper lined petri dishes.

Purples mixed in 50:50 and 75:25 (Neutral Red:Nile Blue A) combinations were most easily distinguished from the standard stains of Neutral Red and Nile Blue A. Neutral Red and Nile Blue A mixed in a 25:75 proportion is indistinguishable from Nile Blue A only. Although it is accepted that Nile Blue A is not passed trophallactically (N.-Y. Su, University of Florida, unpublished data), in order for this combination

TABLE. 1. PERCENT SURVIVAL OF WORKER TERMITES AFTER 5 D OF FEEDING ON STAINED FILTER PAPER AND AFTER 14 D OF FEEDING ON UNTREATED FILTER PAPER.

Treatment	Mean[2] percent survival after 5 d (S.E.)	Mean percent[3] survival after 14 d (S.E.)
Untreated control	95.00 ± 6.0	95.00 ± 0.70
100:0[1]	96.25 ± 0.5	85.00 ± 1.58
75:25	96.25 ± 1.5	88.75 ± 0.48
50:50	92.00 ± 6.0	90.00 ± 0.82
25:75	93.25 ± 5.5	81.67 ± 2.03
0:100	97.25 ± 5.5	87.50 ± 1.04

[1]Proportions in which Neutral Red to Nile Blue A solutions were mixed.
[2]N = 2.
[3]N = 4.

stain to be considered useful for population estimates, additional tests examining possible trophallactic transfer must be conducted. Experiments measuring the longevity of purple color should also be conducted before this stain is used for territory identification if mark-recapture cycles are longer than 14 d due to the possibility of the color fading to red or blue.

SUMMARY

The colors that are created using 0.5% (wt/wt) Neutral Red and 0.1% (wt/wt) Nile Blue A solutions mixed in a 50:50 or 75:25 proportion was consistently distinguishable from Neutral Red only or Nile Blue A only. Purples based on these proportions stained termites for a minimum of 14 d without significant mortality, which is satisfactory for a single mark-recapture cycle. This purple dye-marker would be useful in establishing territories where multiple colonies exist.

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