Fluorescent Spray Paint as a Topical Marker on Subterranean Termites (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Two species of subterranean termites, *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks), were sprayed with fluorescent paint to assess its practicality as a topical marker. In the laboratory, termite survival was not affected by the spray painting treatment. The percentage of termites that retained the paint marker after 2 weeks was lower with *R. virginicus* (33.2%) than with *R. flavipes* (74.7%). The percentage of termites that retained the red paint over time was higher than either the blue or green paints. Of the 6 colors (red, pink, orange, green, yellow, and blue) tested, only 3 color complexes (red-pink-orange; blue; yellow-green) were distinguishable on the termites. This technique was used to map the foraging territories of 7 subterranean termite colonies in the field. The advantages of this technique over standard oil-soluble mark methodologies are discussed.

KEY WORDS: Insecta, *Reticulitermes*, foraging territory, topical marker

INTRODUCTION

Research on the biology and ecology of *Reticulitermes* spp. is complicated by the cryptic life-style of these subterranean insects. Information on the foraging territories of subterranean termites is important in understanding their biology and in determining the efficacy of control tactics. As a consequence, the foraging territories of subterranean termites have been estimated by a variety of methods. King & Spink (1969) and Howard *et al.* (1982a) used direct excavation, which unfortunately prevents further study of population dynamics. Techniques which are less destructive offer the advantage of continuous monitoring of the termite colony. Observations on the aggressive behaviors of termites from different locations also have been used to map subterranean termite foraging territories (Jones 1990). However, conspecific aggression in *Reticulitermes* varies markedly and is not a reliable index for colony separation (Thorne & Haverty 1991).

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Mark-recapture techniques also have been utilized. Spragg & Paton (1980) traced radioisotope-labeled termite foragers, yet this technique is impractical in an urban setting because of the radioactive isotope. Oil-soluble dyes have been used to map termite foraging territories (Lai *et al.* 1983, Su *et al.* 1984, Su & Scheffrahn 1988, and Grace *et al.* 1989). However, all 12 dyes tested by Su *et al.* (1991) provided a concentration-mortality response in *Reticulitermes* spp. In addition, these dyes require that the termites be transported to and maintained in the laboratory until sufficient dye is ingested to differentially color the fat body. This paper reports the results of my work with fluorescent spray paint as a topical marking system for use in subterranean termite foraging territory studies.

MATERIALS AND METHODS

Termite-infested pine logs were collected from the University of Georgia Westbrook Farm in Spalding County, Georgia. Termites were extracted from the pine logs using corrugated cardboard rolls, via the technique of La Fage *et al.* (1983). The extracted termites were maintained in 26 x 19 x 9cm (L x W x H) plastic boxes with moist filter paper and blocks (2 x 2cm) of white pine wood at 23°C in complete darkness for 1 to 4 weeks until used in the tests.

Two species, *Reticulitermes flavipes* (Kollar) and *R. virginicus* (Banks), were tested and termites from at least 3 different colonies were used in each laboratory bioassay. Termites were marked, for the laboratory tests, by placing 50 termites in 100 X 16 mm plastic Petri dishes inside a 66 x 35.5 x 50.8cm (L x W x H) cardboard box. They were then sprayed with Fluorescent Glo Paint (Aervoe - Pacific Co. Inc., Gardnerville, NV). The spray can was held 60cm away from and 30cm above the termites so that the stream of spray paint would drift down on the termites in the Petri dish. The spray nozzle was depressed 3 times for one full second for each group of termites. Verification of termites having received a mark was confirmed by visual inspection, in the dark, using a Versalume Adjusta-Beam Blacklight (Raytech Industries, Stafford Springs, CT).

Three separate laboratory tests were conducted. In the first, marked termites were placed in a 74ml (5 x 3.6cm diam/depth) round plastic container with 20g of autoclaved and oven-dried soil (85% sand, 5% silt, and 10% clay) brought to 15% moisture (w/w) with deionized water and a 2 x 2cm block of white pine. The containers of termites were maintained in an environmental chamber (Percival I-35VL) at 24°C in total darkness. Twenty groups of 50 termites, for each species, were painted with red paint and 4 groups of 50 unpainted termites were used as controls. The number of survivors and the number of marked termites were recorded weekly for 4 weeks by removing all termites from each

container, separating them from the soil, and examining the termites under a blacklight in the dark.

A Petri dish test was conducted to determine if the spray paint mark affected termite survivorship and if the paint could be transfered to unmarked termites. Groups of 50 termites were painted with one of three colors - red, blue or green. The painted termites were placed in a Petri dish (100 x 15mm) containing a 5.5cm diameter #1 Whatman filter paper disk moistened with deionized water and a single $1cm^3$ cube of white pine wood. Fifty unpainted termites, from the same colony, were added to each Petri dish with the painted termites. The termites in each dish were counted and examined under a black light once a week for 4 weeks to determine survival and the number of marked termites. One replicate consisted of 4 petri dishes containing termites from a single colony. Each dish contained termites painted with one of the three colors plus a control of 100 unpainted termites. Both *R. flavipes* and *R. virginicus* were tested and each species and color combination was replicated 4 times.

A color differentiation study also was conducted. Twenty-four groups of 50 termites were sprayed as described above. The colors tested were red (Aervoe #180), pink (#181), orange (#182), blue (#183), green (#184), and yellow (#185). Known numbers of termites, marked with each color, were placed in 100 x 15mm plastic Petri dishes along with unmarked termites. Marked termites of each color also were mixed in various combinations within other Petri dishes. Five different people were then asked to view the dishes of termites under a black light, and record the numbers of marked termites in each dish by color. To aid with color differentiation, a 12.7 x 20.3cm index card marked with the different fluorescent paints was used as a key card in one test. A second test was conducted without a key to the different colors.

In the field, 7 termite colonies: 5 *Reticulitermes flavipes*, 1 *R. hageni* (Banks), and 1 *R. virginicus*, were marked with spray paint for delineation of termite foraging territories. Termites were located using the bait-stake survey method and monitored using a modification of the underground monitoring stations described by Grace *et al.* (1989). In termite monitoring stations the corrugated cardboard was replaced by termite sandwiches. These food and aggregation substrates were constructed of weathered (exposed to the elements for at least 7 months) white pine wood cut into 4 x 12cm (W:H) sections 2mm thick. Each wood section had two 2mm diameter wooden dowels stapled to one side as spacers. Sandwiches were made by combining 10 sections and binding them together with plastic cable ties. Termite sandwiches were removed from a monitoring station, the plastic cable tie cut, and termites knocked onto a 39 x 25 x 2.5cm (LxWxH) aluminum pan by gently

tapping the individual sandwich sections together to record termite activity.

Termite foraging territories were estimated using the spray paint mark. Termites were removed from one monitoring station per site and spray painted at the field site. Termites were marked with spray paint by placing them, excluding dirt and debris, into the bottom of a 100 x 15mm plastic Petri dish (ca. 2g of termites - enough to fill the Petri dish without having termites crawling over one another) inside a cardboard box and spraying them as described for the laboratory trials. Within 5 minutes, the painted termites were returned to their trap of origin. When several monitoring stations within a given area were established, the termites from one station were removed, painted, and returned to that monitor. One week later termites from all the monitors in an area were removed and examined under blacklight for the presence of paint marked individuals inside a "portable darkroom". The portable darkroom was built using 2.5 x 3cm white pine lumber to frame a 35.5 x 66 x 50.8cm (WxLxH) box, which was covered with 6 mill black plastic on 3 sides. The open side of the portable darkroom was covered with a flap of black plastic to allow one person to view the aluminum pan with termites. The total numbers of unmarked and marked termites, from each trap, were counted using an aspirator and a hand-held counter. Those monitors containing at least one marked individual were considered to be part of the same termite colony. If any monitors in that area did not contain paint marked termites, those individuals were sprayed with a different color and returned to their original monitoring station. One week later all monitors within the area were again examined for the presence of marked termites. By the second week, the presence of termites marked with the different colors of paint would establish which termite monitors were utilized by the same colonies. Once the foraging territory of a colony was delineated, all termites from each monitor were returned to the laboratory, marked with Nile Blue A, and the foraging populations of each colony estimated using the triple mark-recapture technique described by Su & Scheffrahn (1988).

A comparison of the movement of termites marked with spray paint and an oil-soluble dye also was conducted in the field. Termites from 2 *R. flavipes* colonies in the same backyard in Lamar County, Georgia were marked with either Nile Blue A or red spray paint. The termites from the colony designated for the spray paint treatment were marked and enumerated as previously described. Those marked with Nile Blue A were collected from the field, returned to the laboratory and maintained for one week in Petri dishes (ca. < 2g termites/dish) in environmental chambers in total darkness at 24°C. Each dish contained a 9cm diameter #1 Whatman filter paper disk stained with 0.03% (w/w) Nile Blue A dye

and moistened with 0.9ml of deionized water. Once a week for 5 weeks, all termite monitoring stations in both colonies were checked for the presence of marked termites. Those termites marked with Nile Blue A were counted under ambient light conditions. The percentage recapture data were recorded for 5 weeks on the aforementioned colonies.

Percentage of survivors and percentage of marked termites from the laboratory study were analyzed using the SAS *t*-test procedure (SAS Institute 1985). The percentage survival data were compared for each termite species by week. The percentage marked termite comparisons were tested for each species between consecutive weeks. The field and color differentiation data were not analyzed.

RESULTS AND DISCUSSION

The effect of allowing the spray paint to drift down on the termites was to speckle the insects with various sized droplets. The paint adhered to all body parts of the termites as spots which were readily identifiable under UV light but were not visible under ambient light conditions on the majority of the marked termites. In the laboratory, all 50 termites were marked with only 3 one-second bursts of paint. In the field, 100% coverage also was achieved with 3 bursts of paint.

Myles & Grace (1991) experimented with spray paints as an adhesive for borate dusts on termites. Although no data were provided, they reported that the spray paints were nontoxic. In my laboratory tests, survival of the termites was not affected by the spray painting treatment in either the Petri dish or soil bioassays (Table 1). By week 4, overall termite survival in both tests was low (soil test *R. flavipes* 34% and *R. virginicus* 13%; Petri dish test *R. flavipes* 66% and *R. virginicus* 52%), presumably due to problems associated with the experimental design. The survival of small groups of termites separated from the parent colony can be affected by numerous factors including container size, matrix within the container, and food supply (Smythe & Carter 1970, Lenz & Williams 1980, Lenz *et al.* 1984). In my tests, however, comparisons of percent of survival between treatments and controls were not significantly different within each test for each week of both bioassays (*t*-test; df 22 & 62; $P < 0.01$).

Two weeks after marking, *R. flavipes* retained more of the red spray paint (88.5%) than did *R. virginicus* (52.1%) in the soil bioassay (Table 2). With the exception of the week 1 to week 2 comparison for *R. flavipes* both termite species lost significantly more paint each week (Table 2). In the Petri dish tests, there was no indication that the paint mark is transferred from one individual to another because the number of marked individuals continuously decreased over time (Table 3). In addition, termites retained the paint marker differentially for the

Table 1. Percentage survival of the original termites marked with fluorescent spray paint compared with unmarked controls by species and week from soil bioassay

Termite Species	Week After Painting	Treatment	Mean Percent Survival	± sd	P>t[a]
		SOIL TEST			
R. flavipes	1	unpainted	88.0	0.03	0.39
	1	painted	85.3	0.12	
R. flavipes	2	unpainted	83.0	0.03	0.84
	2	painted	81.9	0.23	
R. virginicus	1	unpainted	83.5	0.06	0.60
	1	painted	81.5	0.09	
R. virginicus	2	unpainted	62.0	0.13	0.59
	2	painted	57.7	0.14	
		PETRI DISH TEST			
R. flavipes	1	unpainted	96.0	0.05	0.82
	1	painted	97.3	0.08	
R. flavipes	2	unpainted	76.0	0.34	0.64
	2	painted	88.9	0.13	
R. virginicus	1	unpainted	85.9	0.17	0.73
	1	painted	89.5	0.20	
R. virginicus	2	unpainted	73.0	0.10	0.79
	2	painted	75.2	0.18	

[a] Rows followed by an asterisk representing consecutive weeks for each species were significantly different (t-test; df = 22 & 14; $P < 0.01$;)

different paints tested (Table 3). A higher percentage of termites retained the red paint mark than either the blue or green paint (Table 3). The reasons for this differential retention are unknown. It cannot be explained by differences in the formulation of the spray paints because the percentage of solvents used were identical for the various colors

Table 2. Percentage of termites painted with fluorescent paint that retained the mark by species and week from soil bioassay.

Termite Species	Week After Painting	Mean[a] Percent Marked	± sd	P>t [b]
R. flavipes	0	100	0	
				0.0001*
	1	83.3	0.13	
R. flavipes	1	83.3	0.13	
				0.15
	2	88.5	0.09	
R. virginicus	0	100	0	
				0.0001*
	1	72.6	0.11	
R. virginicus	1	72.6	0.11	
				0.0001*
	2	52.1	0.13	

[a]Determined from the number of termites that had survived the previous week.
[b]Rows followed by an asterisk representing consecutive weeks for each species were significantly different (t-test; df = 22; $P < 0.01$;)

tested (Richard Carlson, Technical Director, Aervoe-Pacific Co., Gardnerville, NV, personal communication). Over time, *R. flavipes* also retained more of each type of paint than did *R. virginicus* (Table 3). These differences might be explained by the composition of the cuticular hydrocarbons between the two species (Howard *et al.* 1978, Howard *et al.* 1982b). It also may be possible that differences in grooming behavior are responsible.

Results of the color differentiation tests showed that there are only 3 color complexes which can be distinguished on termites. Termites painted with red, pink, and orange are indistinguishable from one another as are those painted with yellow and green. Blue is easily separated from all the other colors as are the yellow-green complex from the red-pink-orange complex. Use of the color key did not aid color separations beyond the aforementioned 3 color complexes. For example, without the color key, 5 out of 5 people were able to recognize the Petri dish containing only termites painted with red paint. In contrast, while using the color key, only 1 out of 5 recognized the red group (the other 4 recognized pink and orange within that group). Recognizing the yellow or green colors, in contrast to the blue or reds, is made difficult by the fact that both species of termites exhibit a

Table 3. Percentage of termites painted with fluorescent paint that retained the mark by species, color, and week from petri dish test

Termite Species	Week After Painting	Mean ±sem Percent Marked Termites by Spray Paint Color [a]			P>t [b]
		RED	BLUE	GREEN	
R. flavipes	0	100±0	100±0	100±0	
					0.0001*
	1	97.3±0.1	86.3±0.1	51.5±0.1	
					NS
	2	84.5±0.1	78.5±0.1	47.3±2.1	
R. virginicus	0	100±0	100±0	100±0	
					0.0001*
	1	66.7±7.2	55.1±12.4	30.4±7.3	
					0.0001*
	2	40.1±10.2	30.8±15.3	9.7±5.5	

[a] Determined from the number of termites that had survived the previous week.
[b] Rows followed by an asterisk representing consecutive weeks for each species were significantly different (t-test; df = 14; $P < 0.01$;)

natural yellow-green fluorescence under UV light.

In the field, I compared the movement of termites marked with spray paint to termites marked with the oil-soluble dye Nile Blue A. Termites from two separate colonies were marked with either spray paint or Nile Blue A. The mark and recapture data for two *R. flavipes* colonies in the same back yard in Lamar County, Georgia are listed in Table 4. The termites from Monitor #3 (Colony 1) were spray painted whereas the termites from Monitor #1 (Colony 2) were marked with Nile Blue A (Fig. 1). Marked termites were released on the same day. The triple mark-recapture data estimated the foraging populations (mean±SEM) of these two colonies to be 372,992± 72,235 for Colony 1 and 239,973± 15,789 for Colony 2. These data show that termites marked with spray paint were recovered from both monitoring stations at least 5 weeks later. Although these data cannot be compared statistically the percentage recapture of spray paint marked termites is consistent with the termites marked with the oil soluble dye for these two adjacent colonies. Despite the fact that Colony 1 had a higher percentage of the foraging population marked with spray paint (1.5%) than Colony 2 with the fat soluble dye (0.8%) these data would indicate that termite movement within the colony was not disrupted by the topical paint mark when compared to an internal marker.

I have used spray paint marking to estimate the foraging territories of 7 subterranean termite colonies of 3 species: *Reticulitermes flavipes*,

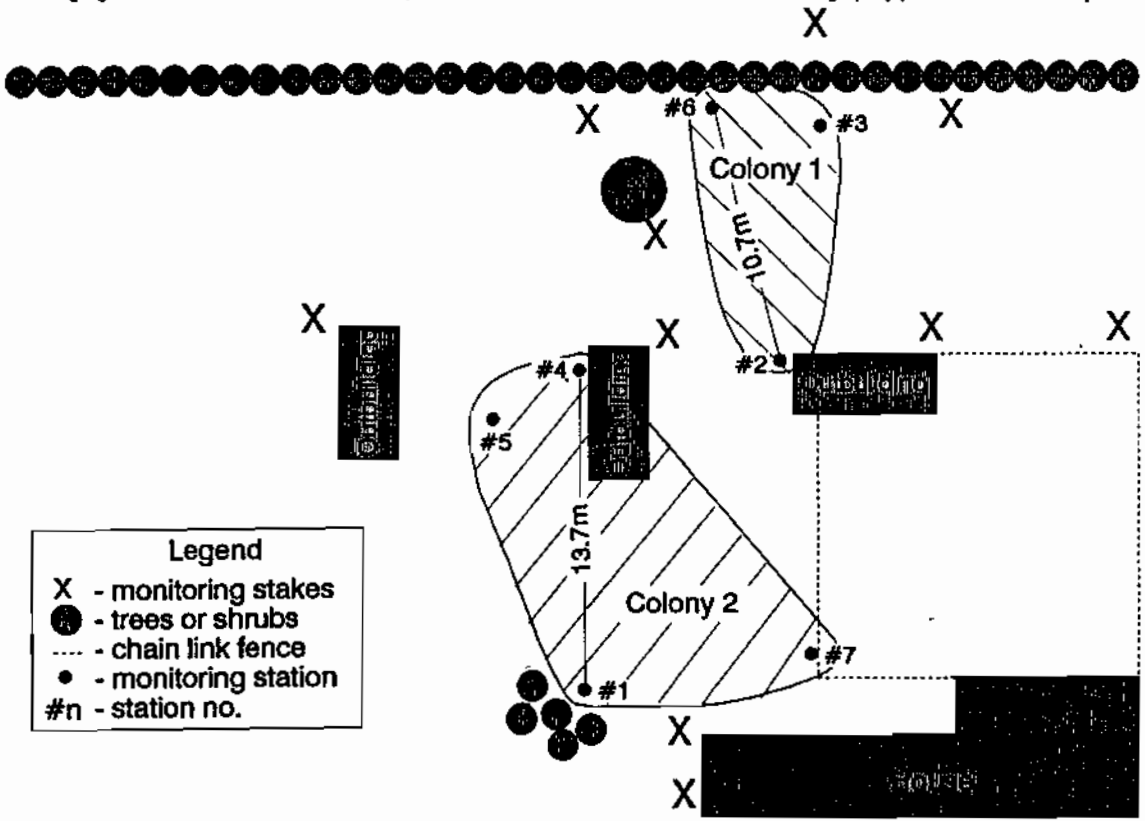
Table 4. Numbers of termites collected from one field site in central Georgia using spray paint or oil-soluble dye mark-recapture techniques on two colonies of *Reticulitermes flavipes*

WEEK POST MARKING	TRAP NUMBER	NUMBER TERMITES CAPTURED	% MARKED
		SPRAY PAINT	
0	#3	5788	100
	#2	368	0
1	#3	580	13.8
	#2	3322	2.0
2	#3	2768	4.3
	#2	1137	1.8
3	#3	1512	2.4
	#2	895	2.0
		NILE BLUE A	
0	#1	1985	100
	#4	114	0
	#5	2058	0
1	#1	1634	9.2
	#4	176	4.0
	#5	129	1.6
2	#1	1902	5.0
	#4	622	2.3
	#5	440	1.6
3	#1	738	3.8
	#4	1521	0.3
	#5	1376	0.6

R. virginicus, and *R. hageni*. The size of the foraging territories of these colonies ranges from 42m² for a *R. flavipes* colony to a linear distance of 1.2m between two monitors for a *R. virginicus* colony. In the field, *R. flavipes* retained the paint mark longer than the other two species. After 4 weeks, none of the *R. virginicus* or *R. hageni* recovered from monitored colonies were marked with paint. This is consistent with the laboratory work that showed *R. virginicus* lost the mark faster than did *R. flavipes*.

The loss of the spray paint mark over time makes this technique unsuitable for estimation of termite foraging populations. For the purpose of estimating the foraging territories of *Reticulitermes* colonies, however, the loss of the paint mark is not important because the

Fig. 1. Map of foraging territories of *Reticulitermes flavipes* colonies from termite movement test using spray paint or oil soluble dye in Lamar Co., Georgia[1]



[1]Termite monitoring stations are numbered in chronological order of establishment. Only monitors 1-5 were active at time of termite movement test.

presence of one marked termite in a monitor is sufficient to determine common use between monitors. The fact that the paint mark is not transferred between termites allows its use in determination of foraging territories. In conclusion, the topical spray painting technique offers several advantages over internal (oil-soluble dye) markers. First, the mark can be applied to termites in the field and the termites returned directly to the colony with a minimum of separation. Second, this technique can be used in conjunction with the oil-soluble dyes to maintain records of territory shifts between adjacent colonies within an area during population studies. Third, the spray paint colors, when viewed under UV light, are unnatural and not easily mistaken for any naturally occurring termite coloration. An additional advantage of spray paint marking is that there are several colors available for use in the field. When several active termite traps have been identified within an area, the advantage offered by several marking colors is apparent if delineation of separate colonies and their concomitant foraging territories is the goal.

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