Use of Dyed Matrix in Bait Stations for Determining Foraging Territories of Subterranean Termites (Isoptera: Rhinotermitidae: *Reticulitermes* spp. and Termitidae: *Amitermes wheeleri*)

by

Thomas H. Atkinson[1]

ABSTRACT

A method is described for determining foraging territories of subterranean termites in the field through the use of a bait matrix impregnated with dye markers (Nile Blue A, 0.1% wt/wt; Neutral Red, 0.5% wt/wt) placed inside commercial monitoring/baiting stations (*Sentricon*® *Termite Colony Elimination System*) or bucket stations. The method is useful at field sites for research related to the inter-colony interactions or baiting systems where the identity of different colonies and their foraging territories is important. The technique has been validated for *Reticulitermes flavipes* (9 colonies), *R. virginicus* (1 colony), and *Amitermes wheeleri* (2 colonies) in Texas.

Termites readily feed on the dyed matrix in the field and quickly acquire a very visible mark, which may persist for long periods. Dyed termites move quickly to other stations that are part of the same foraging territory (30-90 days). Based on spatial and temporal patterns of dye markers superimposed on the overall pattern of activity at study sites, termites bearing the dye markers are apparently moving throughout the entire foraging territory of individual colonies.

Unlike standard mark-release-recapture methods this technique does not permit an estimate of numbers of foraging individuals in the colony. Advantages of this method are:

1. Handling of termites is reduced to a minimum.
2. Access to a laboratory is not required.
3. The process is less labor intensive than mark-release-recapture techniques.
4. Site visits can be kept to monthly intervals (or longer).
5. With the exception of the specially prepared dyed matrix, the procedure can be done entirely with the use of standard commercial components or with a variety of previously described monitoring systems.

---

[1]Dow AgroSciences, 1221 South Congress Ave., No. 913, Austin, TX 78704 email: thatkinson@dowagro.com

®Trademark of Dow Agro Sciences LLC

6. The process is simple enough that non-scientists could perform it with adequate training in the use of baiting systems.

7. The technique can be easily integrated with a variety of experimental protocols, especially those that do not involve active ingredients, without a significant increase in effort.

A field preference test confirms laboratory data showing that Nile Blue A is a feeding deterrent to termites when offered in close proximity to stations containing an identical matrix lacking the dye. Nonetheless, appreciable feeding was detected at all sites in the field indicating that matrix with Nile Blue A is still a palatable food source by comparison with existing sources of structural and naturally occurring wood.

Key words: Sentricon, Nile Blue A, Neutral Red, dye markers, subterranean termites, *Reticulitermes flavipes*, *R. virginicus*, *Amitermes wheeleri*, foraging territory, feeding deterrence, palatability

INTRODUCTION

Dye markers for studying the ecology of subterranean termites colonies is a relatively recent technology (Esenther, 1980; Lai *et al.*, 1983). In almost 30 years of work a total of 3 dye markers have been found that combine persistent marking with acceptably low levels of mortality: Neutral Red, Sudan red, and Nile Blue A (Su *et al.*, 1991; Oi & Su, 1994; Evans, 1997). One of the most important uses of dye markers has been to estimate foraging territories of specific colonies and to distinguish foraging sites when multiple colonies are present at the same study area. In an important sense, the modern phase of the study of subterranean termite ecology dates from the development of this enabling methodology. The ability to actually study the impact of a particular bait toxicant or baiting system on specific subterranean termite colonies was not possible prior to the development of these techniques.

The basic method that has evolved and been validated for a variety of species consists of the following steps (Su *et al.*, 1988; Su & Scheffrahn, 1988; Grace & Abdallay, 1989; Grace *et al.*, 1989; Jones, 1990a; Jones, 1990b; Su *et al.*, 1993; DeMark *et al.*, 1995; Haagsma & Rust, 1995; Forschler & Ryder, 1996; Evans *et al.*, 1998):

1. Monitors such as stakes, boards, rolls of paper, etc. are positioned in an area where subterranean termite foraging is suspected in some sort of basic grid (not necessarily rectangular).

2. As termites find individual monitors, a bucket station (or variation) is established at the site of an active monitor. After a variable (and unpredictable) amount of time, multiple bucket stations have been established.

3. Termites are carefully removed from a single station and brought into the laboratory where they are separated from dirt and debris. They are then force-fed paper impregnated with a dye marker.

4. Marked termites are returned to the field, usually after 1 week and released into the original station from which they were removed.

5. During subsequent visits all stations are inspected. Marked termites may be recovered from stations other than the original release station, demonstrating that underground connections exist. Steps 3 and 4 may then be repeated at other stations from which marked termites are recovered.

6. An estimate of foraging population sizes can also be calculated with this method by counting the number of individuals released and the numbers of marked and unmarked individuals captured on subsequent visits.

This process is labor intensive and requires a long lead time until enough activity has developed in monitoring stations at a site before the actual marking process can begin. It also requires access to a laboratory and is logistically difficult to carry out at remote field sites. A variation on the technique involves the use of fluorescent spray paints (Forschler, 1994) which requires most of the steps outlined above and still involves extensive handling of the termites. The paint marker is often not visible on termites in daylight and requires a completely darkened room or chamber and a black light. Evans (1997) showed that paint particles are rapidly groomed off by the termites after applications.

Many candidate dyes have been tested against a variety of species (Esenther, 1980; Lai, Tamashiro *et al.*, 1983; Su *et al.*, 1983; Su, Scheffrahn *et al.*, 1988; Salih & Logan, 1990; Su, Ban *et al.*, 1991; Oi & Su, 1994; Evans, 1997). In general dyes have been sought that were persistent for long periods, not transferred among nestmates after an initial exposure period, and had no deleterious effects on survival or behavior. Most dyes tested to date are either toxic, non-persistent, or both. These criteria are necessary to meet the basic requirements of mark-recapture studies such as integrity and persistence of marks. The use of ingested dye markers for population estimates has been challenged recently on biological and statistical bases (Forschler & Townsend, 1996; Thorne *et al.*, 1996; Curtis & Waller, 1997; Evans, Lenz *et al.*, 1998). Despite the caveats, there are still no suitable alternatives at present.

While the criteria of persistence and lack of transfer are important for population estimates, they need not be met to determine foraging territories of individual colonies in the field. There are many studies where the identity and location of foraging activity of a colony is

important in its own right, regardless of the actual colony population size. In any event, it may be just as important to document the activity and foraging territories of colonies adjacent to the one actually baited as demonstrated in a recent study by Tsunoda *et al.* (1998). Their work highlights the complications that may arise at sites known to have multiple colonies present in close proximity. The authors only marked (with Nile Blue A) the colony that was later baited. Their study results remain ambiguous because they could not determine whether the termites found inside the former foraging territory of the baited colony were from neighboring colonies (known to be active in close proximity prior to baiting) or represented a resurgence of the baited colony. The authors concluded that mark-release-recapture methods have limited applicability for determining colony elimination. This conclusion does not necessarily follow. Delimitation of the foraging territory of non-baited colonies with a different dye marker (e.g., Neutral Red) would have eliminated the confusion

Given the complexity of standard mark-recapture techniques, especially at remote study sites without access to laboratory facilities, I decided to test the idea of directly introducing the dyed matrix into commercial baiting stations of the Sentricon System. If effective, it would considerably reduce the labor involved in identifying distinct colonies present at the same site.

During 1997 and 1998, I used specially prepared bait matrix impregnated with dye markers at a number of study sites in Texas. Important questions were:

1. Would subterranean termites consume dyed matrix under field conditions?
2. Would termites be visibly dyed under field conditions?
3. Would termites move from stations containing dyed matrix, and if so, how far?
4. How long would this process require?
5. Would movement of dyed termites correspond with the extent of foraging territories of subterranean termites as reflected by the monitoring stations?
6. Would consumption rates of dyed and undyed matrix differ under field conditions?

An additional objective was to determine whether the consumption rates of dyed and undyed matrix differ under field conditions.

MATERIALS AND METHODS

Field sites

All fieldwork was done at 9 sites in Texas with colonies of *Reticulitermes*

spp. (*R. flavipes* (Kollar) and *R. virginicus* (Banks)) and *Amitermes wheeleri* (Desneaux) from January 1997 until December 1998 (Table 1). At most sites, Sentricon in-ground monitoring stations (Dow AgroSciences LLC, Indianapolis, IN) were placed around the perimeter of structures at approximately 3m intervals according to standard label instructions. With the exception of one commercial office building (Woodlands), all were freestanding, single family residences. At most of these sites a substantial number of additional stations were placed near fence lines, property lines, and other places where termite activity was suspected. The Flower Mound and Corpus Christi I sites were set up as rectangular grids of monitoring stations on 3m centers in areas without structures. All sites were established primarily for research purposes and control of structural infestations was a secondary concern and was only pursued after research objectives had been met.

In commercial installations of the Sentricon System, stations are installed in the soil without active bait. Stations contain wooden monitoring devices and are checked monthly to quarterly for signs of termite activity to detect termite activity . When foraging termites have located a station and are present inside it, a Baitube device (0.5% hexaflumuron on a cellulosic matrix (Recruit® II) inside a slotted plastic

Table 1. Study sites in Texas for use of dyed matrix for determining foraging territories. Dyes Used: blue = Nile Blue A (0.1% wt/wt); red = Neutral Red (0.5% wt/wt). At "grid" sites, monitoring stations were placed on 3m centers in a rectangular grid. At "structure" sites, station were placed around perimeter of structure as well as in other locations, but not in regular patterns. At sites where the structures were infested, colonies were eliminated using Recruit II® after dyeing.

Site	Colony	Species	Dye Used	date dye installed[a]	last date checked	Type site	Structural Infestation?
Flower Mound	1	*R. flavipes*	blue	01/29/97	ongoing	grid	No
	2	*R. flavipes*	blue	02/20/98	ongoing	grid	No
Coppell I		*R. flavipes*	red	08/03/98	ongoing	structure	Yes
Coppell II		*R. flavipes*	blue	07/14/98	ongoing	structure	Yes[c]
Bryan		*R. flavipes*	blue	05/19/97	ongoing	structure	Yes
Navasota		*R. flavipes*	blue	11/13/97	ongoing	structure	Yes
Woodlands		*R. virginicus*	red	07/30/98	ongoing	structure	Yes
Corpus Christi I	1[b]	*A. wheeleri*	blue	07/28/98	ongoing	grid	No
	1[b]	*A. wheeleri*	red	01/26/99	ongoing	grid	No
	2	*R. flavipes*	blue	07/01/98	ongoing	grid	No
Corpus Christi II	1	*R. flavipes*	red	07/28/98	12/01/98[d]	structure	Yes
	2	*R. flavipes*	blue	03/26/98	12/01/98[d]	structure	yes
Corpus Christi III		*A. wheeleri*	blue	07/01/98	12/01/98[d]	structure	No

[a] Does not correspond to date when site first established.
[b] Same colony treated with second dye after 6 months.
[c] Structure not actually infested, but colony active around foundation and later eliminated.
[d] Sites discontinued.

tube) is substituted for the monitoring device (Su *et al.* 1995a). Termites are introduced into Baitube* devices using the Self Recruitment* procedure (Su *et al.* 1995a; Su, Thoms *et al.* 1995). Termites are carefully collected from monitoring devices and placed inside the upper chamber of the Baitube device where they begin feeding and help reestablish connection with foraging tunnels in the soil. Normally additional stations (auxiliaries) are placed in the close vicinity (<45cm) of active stations.

During the period of dye marker evaluation, all active stations were typically baited with blanks. At some sites bucket stations (Su & Scheffrahn, 1986) containing monitoring blocks of MD499 were also used. "Board" stations (2cm thick x 15 x 15cm MD499 boards placed on the soil surface) were also used at Corpus Christi sites where species of *Amitermes* were found (*Amitermes* spp. were also present inside standard soil stations). These "non-commercial" stations were placed as auxiliaries to standard Sentricon stations with termite activity

Baitube devices were specially made without active ingredient (same matrix as Recruit II) and with Neutral Red (0.5% wt./wt.) or Nile Blue A (0.1% wt./wt.) dyes. In most cases, termites in active Sentricon stations were recruited to blank Baitube devices (no A.I. or dye) using the Self Recruitment procedure when activity was detected. On the few occasions when dyed matrix was introduced in a bucket or board station, the matrix was removed from the tube, moistened, and placed between the infested wood and the soil.

Typical procedures are shown in Fig. 1. In this example stations are on a grid pattern and some data about termite activity in stations is known. A dyed Baitube device is placed inside a single station with active termites using the Self Recruitment process (A). On a subsequent monitoring date, not necessarily the next, dyed termites are recovered from a different station. This indicates that the termites foraging inside the original release station are of the same colony group as the new station. Dyed matrix may be optionally placed inside other stations in which dyed termites are recovered (C). In general, Baitube devices containing dyed matrix are replaced when more than 50% of the matrix has been consumed. At some point, generally several months after initial dye placement, no dyed termites are recovered from new stations, while dyed termites are still found in stations where previously noted (D).

While foraging territories are generally stable for the short and medium term in a general area (months to years), foraging activities are dynamic (Fig. 1, E, F). The same stations may not always remain active and foraging may shift to new stations (E). Likewise, interactions

In the original publication an error was made in printing Fig. 1. The corrected version appeared in a later issue, hence the page number out of sequence

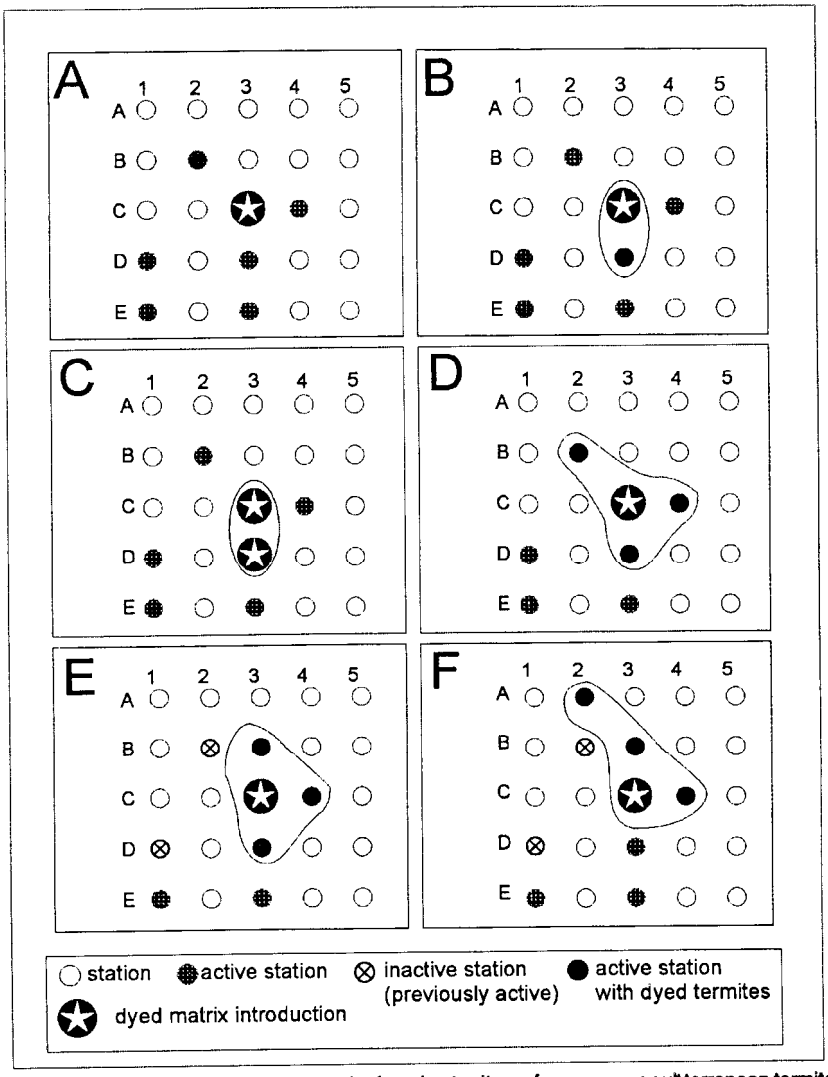

Fig. 1. Basic methodology for delimiting the foraging territory of one or more subterranean termite colonies. A. Several stations in a grid have termite activity. Dyed matrix is placed into a single station, c3. B. Later dyed termites are found in another station, d3. More dyed matrix may be added to the original release point, c3. C. Dyed matrix may be optionally added to the station from which dyed termites were recovered, d3. D. Eventually no further spread of dye is detected. There may be other active stations at the site (d1, e1, e3) E. Over time some stations may become inactive (b2, d1). F. Other stations may show new activity (with (a2) or without dye marker). Specific stations may be contested by colonies in close proximity as evidenced by presence or absence of marked individuals (d3).

between adjacent colonies may shift slightly along encounter zones (F).

Feeding preference

A field choice test was set up in the fall of 1997 at a site in Navasota, Texas. A ring of 6 Sentricon stations were placed around 2 existing stations that had shown consistent activity as auxiliary stations. Stations were no more than 45cm from each other or from the central station. Baitube devices containing either blank matrix or the matrix containing 0.1% (wt./wt.) Nile Blue A were assigned randomly to active auxiliary stations following normal recruitment procedures. The first group of replicates were placed on September 19, 1997 and removed on October 16. At one of the 2 groups, termite activity was very high and another group of dyed and undyed tubes were placed in the same stations but with the treatments reversed (blank Baitube devices were placed into stations which previously contained Baitube devices with Nile Blue A, and vice versa). These were removed on November 13. The matrix was removed from the Baitube devices and air dried at ambient conditions prior to weighing.

RESULTS

Field sites

Data for feeding, bait acceptance, and marking are shown in Table 2 (9 colonies of *R. flavipes*, 1 colony of *R. virginicus*, and 2 colonies of *Amitermes wheeleri*). The colony of *A. wheeleri* at Corpus Christ I was dyed twice, first with Nile Blue A, then 6 months later with Neutral Red. In both cases the observed movement of dyed individuals was identical. Termites readily consumed the dyed matrix, in some cases completely emptying out the Baitube devices. Total dyed matrix consumed ranged from 8 – 71g. There was no obvious correspondence between amount of dyed matrix consumed and size of foraging territories as indicated by number of stations with dyed termites or maximum distance from the release point at which dyed termites were recovered.

In all but one case, dyed termites were seen inside the original release station at the next monitoring visit after the introduction of dyed matrix. In 10 of 13 cases, dyed termites were observed inside another station on that same visit. Individual marked termites can cover long distances in a short period. In colony 2 (Corpus Christi I site, *R. flavipes*) individuals marked with Nile Blue A were detected 30m from the original introduction site after 27 days. At the Coppell I site, dyed *R. flavipes* were detected in 6 additional stations after 25 days up to 10m from a single release point. In general, speed of movement seems most related to soil temperature, which influences both bait consumption and individual

Table 2. Consumption of dyed matrix, movement of dyed termites, and persistence of dye markers in soil stations. Dyes Used: blue = Nile Blue A (0.1% wt/wt); red = Neutral Red (0.5% wt/wt.)

								Days after dye introduction			
Site	Colony	Species	Dye Used	dyed matrix cons. (g)	No. stations with dye cons.	No. stations with dyed termites[a]	Max. distance (m) dyed termites moved	dyed termites observed in release station	dyed termites observed in other stations	all dyed matrix removed	last observation of dyed termites
Flower Mound	1	*R. flavipes*	blue	71	2	9	10	89[c]	89[c]	236	653
	2	*R. flavipes*	blue	45	2	6	10	39[c]	109	581	581
Coppell I		*R. flavipes*	red	14	1	11	8	25[c]	25[c]	25	91[d]
Coppell II		*R. flavipes*	blue	24	1	3	10	38[c]	38[c]	77	111[d]
Bryan		*R. flavipes*	blue	17	1	2	5	31[c]	31[c]	31	31[d,e]
Navasota		*R. flavipes*	blue	12	1	2	3	59[c]	59[c]	59	118[d]
Woodlands		*R. virginicus*	red	54	1	2	9	34[c]	34[c]	76	221[d]
Corpus Christi I	1[b]	*A. wheeleri*	blue	12	2	4	10	35[c]	35[c]	247	399
	1[b]	*A. wheeleri*	red	na[f]	1	4	10	65	183	65	217
	2	*R. flavipes*	blue	32	4	10	30	27[c]	27[c]	426	426
Corpus Christi II	1	*R. flavipes*	red	10	1	7	15	35[c]	35[c]	35	35[d]
	2	*R. flavipes*	blue	38	1	2	<1	62[c]	97	159	159
Corpus Christi III		*A. wheeleri*	blue	8	1	1	<1	27[c]	27[c]	27	27[e]

[a] Other than station in which dye was originally released.
[b] Same colony treated with second dye after 6 months.
[c] First visit subsequent to dye introduction.
[d] Colony eliminated with Recruit II.
[e] No further attempts to trace dyed termites.
[f] Dyed matrix consumption not evaluated.
[g] Dye introduced from November-March. Otherwise, dye introduced May-August.

movement. Typically, dye introduced during summer months reached its maximum dispersion within a foraging territory within 30-60 days, while introductions in winter took longer.

Many termites recovered from a station containing a Baitube device with dyed matrix show little or no signs of the dye marker and those that do are frequently not strongly colored. This is quite different from the intense coloration often acquired when groups of termites are force-fed dyed filter paper in the laboratory. Most coloration is in the abdominal region, less commonly in the thorax or head. The lack of intensity suggests that foragers are spending relatively little time inside a Baitube device and that most individuals recovered within a given Baitube device have not spent much time there previously. When termites are force-fed dyed filter papers in the laboratory there are no alternative food sources and no harborages. On two occasions (once for each dye marker) small groups of *Reticulitermes flavipes* were found with intense coloration where the termites originally placed inside the Baitube device presumably had not escaped or been joined by other colony members and had been confined on dyed matrix for a full month. In one of these instances, after 1 month a small group of 50 termites, stained intensely blue, was found inside the recruitment chamber at the top of the Baitube, while mostly non-marked individuals were found feeding at the bottom of the Baitube device where they had clearly entered through one of the lower slots from the soil.

Some individuals that have fed on dyed matrix may not be detectable if most of the termites inside active stations are not extracted and carefully inspected in the field. However, the mark is clear and unambiguous when visible and it is quite simple in practice to recognize it in the field when a white, opaque collecting tray is used and the bulk of the termites present inside a station are emptied out for inspection. In other words, the technique is conservative because it may yield false negatives (dyed termites may not be detected and observer concludes that stations are not linked), but not false positives (error in identification where observer counts an undyed termite as showing dye marker and concludes that stations are linked). When a large amount of dye is delivered to a termite colony over an extended period, the dye is maintained at visible levels for extended periods. Although "persistent" dye markers may fade in the field due to factors identified by Thorne *et al.* (1996), as long as dyed matrix remains available, its is continuously being "pumped" into the colony. The dry weight of a 9cm diameter filter paper (Whatman #1) after impregnation with Nile Blue A or Neutral Red for a wt./wt. concentration of up to 0.5% ranges from 0.492-0.536g (King 1999). The dry weight of the matrix within Baitube devices used

here was approximately 20g. The sheer amount of dye introduced into a colony by direct feeding is potentially 1-2 orders of magnitude greater than that introduced by mark-release methods. When such a large volume of dye has been ingested, it is also highly likely that a very high percentage of the total colony has been exposed.

Soldiers tend to pick up the most intense coloration and apparently retain it the longest. With the exception of Evans (1997), most studies on dye markers have ignored soldiers. Clearly, soldiers are receiving the dye marker via trophallaxis showing that transfer is possible at least during the period when foragers have access to dyed matrix. Previous studies (Lai *et al.* 1983; Su *et al.* 1991) have stressed that there is no transfer of dye from marked individuals to unmarked individuals. This is certainly true when the force-fed termites are allowed to purge their guts prior to being mixed with undyed workers. This is unlikely to be true when forging termites are allowed to move freely. There are several alternative explanations why soldiers retain color longer, none of which can be addressed by the current study. One possibility is that the dye is easier to see since soldiers are directly fed by workers and tend not to have dark gut contents. It is also possible that individual soldiers tend to stay longer at a foraging site than individual workers and therefore tend to accumulate more dye (i.e., stationed on the frontier). Soldiers may live longer than workers and retain dye longer for that reason. A final possibility is that fat reserves (and retained dyes) are more actively metabolized by workers, possibly mobilized during molting, and would tend to disperse faster, independently of survival. This study was not designed to specifically test for longevity of dye markers. At several sites soldiers with visible coloration from Nile Blue A have been detected 1 year (Flower Mound #1) or longer after the last introduction of dyed matrix and at least 10 months after exposure to Neutral Red (Atkinson, unpublished). In several cases (Flower Mound colonies 1 & 2, Corpus Christi I colonies 1 & 2, and Corpus Christi II colony 2) dyed matrix was continuously renewed over long periods up to 581 days, with no apparent ill effects on colony activity. This allows following the foraging activity of a given colony over long periods because the dye marker can be continuously be replaced.

The question arises of whether or not the maximum observed movement of dyed termites actually corresponds with the foraging limits of the colonies tested, as reflected in active monitoring stations. In 11 of the 13 colonies tested, I believe that such did occur. At the Corpus Christi III site, both the original release station and the station where dyed termites were recovered became inactive and the site was discontinued shortly afterwards. At the Bryan site no attempt was made

to follow dye markers beyond a 38 day period during which dyed termites were recovered at a distance of 10m.

The following scenarios are consistent with the hypothesis of dye movement throughout the colony foraging territory (shown diagrammatically in Fig. 2):

1. In some cases, all active stations at the site show the dye marker (Fig. 2, A). Specific examples include *Amitermes wheeleri* at Corpus Christi I with both dye markers, *R. flavipes* at Corpus Christi I, and *R. flavipes* at Navasota.

2. At some sites different dye markers were introduced in stations of what were suspected to be different colonies based on spatial patterns. At the Corpus Christi II site, "red" and "blue" colony territories were separated by 15m at the closest point and remained stable for over 6 months.

3. At other sites, the extent of dye movement has remained stable for long periods without changing (Fig. 2, C and D). At the Flower Mound site (colonies 1 & 2, both *R. flavipes*) are separated by approximately 25m. There are several clusters of active stations in intermediate areas that have never shown any blue dye. Matrix containing Nile Blue A was introduced into colony 1 in early 1997. Dyed matrix was continuously replaced for 236 days with no further spread of dyed individuals after 3 months. The same dye was introduced into colony 2 at another station with activity of *R. flavipes* at the opposite end of the grid approximately 1 year later and reached its maximum spread after 4 months. Both colonies are still active in monitoring stations 2.5 and 1.5 years later, respectively with no sign of further spread of dye. In the meantime, Neutral Red has been introduced at other release points in the grid with no movement to stations within the maximum observed foraging territory of either colony.

4. Introduction of Recruit II bait containing the active ingredient hexaflumuron was applied to stations of a single colony as determined by recovered dyes (Fig. 2, E and F). In these cases the extent of dye movement coincided with the extent of mortality following baiting. In other words, active stations where no dye marker or a different dye marker was found did not become inactive after baiting (Coppell 1, Coppell 2, Woodlands, Corpus Christi II, colony 1). All activity ceased in stations originally occupied by the eliminated colony.

Some authors have questioned the basic assumptions of mark recapture studies using the same dye markers used here for statistical estimates of foraging population (Forschler & Townsend 1996; Thorne, Russek-Cohen *et al.* 1996; Curtis & Waller 1997). To this point there have been no published challenges to the concept that spread of dyed

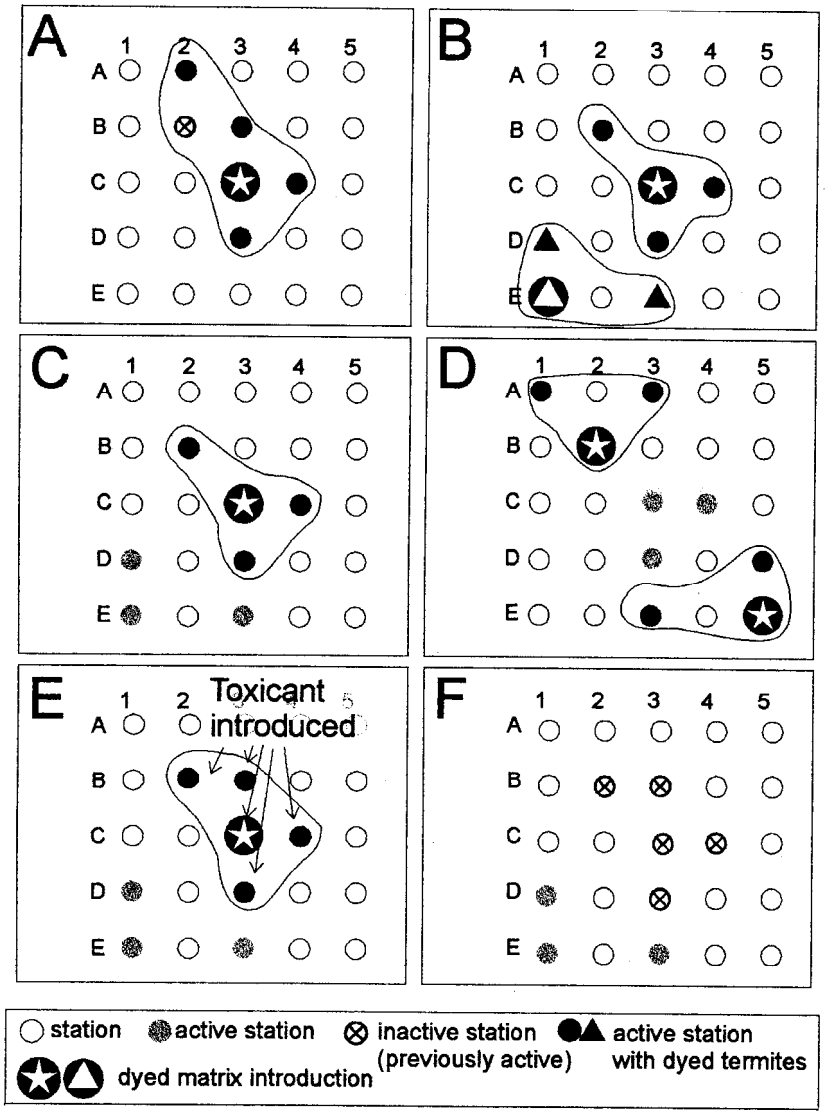

Fig. 2. Observations supporting supposition of dye movement throughout colony. A. All stations currently or formerly active at a site show dyed termites. B. Releases of different, distinguishable dyes form non-overlapping patterns. C. Pattern of dye appearance is stable over periods of several months or longer. Stations from which dyed termites were never recovered are presumably from one or more other colonies. D. A variation on C is when the same dye is released at a second point, e5, after no further dye movement is detected from the first release point, b2. and there are active stations between where dyed individuals have never been seen. E and F. Matrix containing toxicant is introduced into some or all stations from which dyed termites were observed. Cessation of activity has no short term effect on activity in stations from which dyed termites were not found.

individuals indicates colony foraging territories. In any event our ability to delineate a colony foraging territory is dependent on the degree to which a monitoring grid overlaps the entire foraging territories of the colonies under study.

Feeding Preference

Field data (Table 3) confirm laboratory studies (King, 1999) which documented that Nile Blue A is a feeding deterrent. In this study stations containing Baitube devices with the blue dye were in close proximity to those containing blank Baitube devices and termites were completely free to move among them. This suggests that the intensity of natural recruitment processes by foragers, whether in terms of the amount of trail pheromone deposited or other behaviors, depends on the perceived quality or palatability of the alternative food sources. Because individual Baitube devices were not weighed prior to being placed in the field, data are expressed in terms of weight of matrix remaining rather than weight consumed.

Table 3. Comparison of feeding by *Reticulitermes flavipes* on Baitube devices containing an untreated cellulosic matrix with or without the addition of Nile Blue A. Percent consumption based on average weight of 6 blank Baitube devices held under identical drying conditions (21.055g).

Treatment	n	Av. Weight remaining matrix (g)	95% CI	% consumed
0.1% Nile Blue A	10	16.7	±0.90	20.72
Blank	9	9.9	±2.30	52.98

DISCUSSION

The idea of adding the dye directly to the matrix in the field is not new, although I am unaware of published instances where it has been used specifically for the purpose of delimiting territories. Su *et al.* (1988) added paper towels containing 4% (wt./wt.) Sudan red 7B to monitoring stations from which marked individuals had been previously detected using a standard laboratory-based mark-release-recapture technique. In this particular study the extent of the colony foraging territory was already known and the dyed matrix was added to determine the speed and extent of dye movement throughout the colony in the presence of other food sources in the field.

The results of this study confirm that the 2 dye markers in common use for mark recapture studies with subterranean termites of the genus *Reticulitermes*, Nile Blue A and Neutral Red, are suitable for delimiting colony foraging territories when introduced into soil stations for termites to feed on. Although the marks do fade, soldiers from colonies

marked with both Nile Blue and Neutral Red remain detectable for up to 1 year after the last date of dye introduction. Even though dyes may fade, it is possible to continuously replenish dyed matrix for extended periods to maintain marking.

The technique avoids many problems of mark-release-recapture techniques and paints as previously practiced.

1. As described here, it uses standard Sentricon System components (except for special Baitube devices with dyed matrix). There is no need to switch to bucket traps to get a large sample of termites for force-feeding in the lab. In practice the method could be applied using any monitoring/baiting station in which the feeding source can be readily changed. The method might also be modified to use other bait matrices after appropriate testing.

2. Handling of termites is kept to a minimum. There is no need to paint or carry termites to and from the lab.

3. The process can be used where there is no laboratory access, such as at commercial field sites.

4. There is no need to interrupt a monthly schedule, with supplementary visits to release marked termites or to census marked individuals.

5. There is no need for special equipment such as blacklights or spraying containers.

6. The technique can be easily integrated with a variety of experimental protocols without a significant increase in effort.

Some limitations of the technique include:

1. If Baitube devices are used at any stations thought to be part of the same colony (whether blank, dyed, or containing active ingredient), these must be emptied out completely to search for marked individuals. This would be true of any sort of station in which termites are allowed to tunnel into the feeding substrate. This increases the disturbance and may be incompatible with some study protocols. Even so, all work can be done in the field, at the station, and relatively quickly.

2. Because Nile Blue and Neutral Red at the rates used here actually deter feeding, care must be used not to place more palatable food sources in close proximity to stations containing dyed matrix.

3. There is no estimate of foraging population.

Overall, the main advantage this technique offers is that it allows identification of the number of colonies present at a site and their respective foraging territories. This is very useful for field studies, especially at long-term sites, because it offers the possibility of applying treatments at the colony level, rather than at a "site" which may consist of several distinct colonies. This in turn offers the advantage of accounting for inter-colony variation and its significance with respect

to active ingredients, system components, and feeding preferences. Studies on the impact of bait-toxicants on individual colonies often incorporated one or more population estimates (Jones, 1991; Su, 1994; DeMark, Benson *et al.*, 1995; Su *et al.*, 1995a; Forschler & Ryder, 1996; Grace *et al.*, 1996; Su & Scheffrahn, 1996a; Su & Scheffrahn, 1996b; Benson *et al.*, 1997; Haagsma & Bean, 1998). It is useful to know as much as possible about a colony prior to application of a bait toxicant, but it is not necessary to estimate population size to demonstrate efficacy if the end result is cessation of all termite activity in the area of the baited colony. While there may remain questions about the biological and statistical significance of population estimates based on individuals marked with ingested dyes, in actual practice it is statistically and biologically impossible to obtain confidence limits around a population of zero, the desired end point.

Recent studies (Oi & Su, 1994; Evans, 1997) have focused on identifying new dye markers that produce persistent marks without producing mortality, but have not found any candidates which meet both criteria. Both studies identified several candidates that leave visible, though not particularly persistent, marks without increasing mortality. These dyes are not suitable for conventional mark release techniques because the marker is steadily lost after the termites are returned to the field (and probably susceptible to transfer among individuals). They might be useful in the technique described here, especially if introduced from several stations, because the mark is continuously renewed from further feeding and being recirculated via trophallaxis. In many cases a non-persistent marker would even be more desirable because it would allow the relatively few persistent markers to be used subsequently for more complete mark-release-recapture studies for population estimates.

In large part, the effectiveness of this technique depends on the "competitive" palatability of the dyed matrix in the field where colonies have access to other food sources. Recent laboratory research (King 1999) has shown that dyes used in this study, Nile Blue A and Neutral Red, can deter feeding when termites are given a choice between dyed and non-dyed food sources. However, in his study a significant portion of foraging termites was still marked by the dyes even with a strong palatability differential. This is consistent with the results shown here that even though feeding deterrency was demonstrated for Nile Blue A in the field, significant consumption and marking still occurred.

ACKNOWLEDGMENTS

My thanks to Dennis Wujek for preparing the dyed baits. Ed King,

Ellen Thoms, Jamey Thomas, Mike Chambers, and Sterett Robertson provided useful criticism of the manuscript.

REFERENCES

Benson, E.P., J.K. Mauldin & E.S. Bordes. 1997. Evaluation of subterranean termite baiting strategies at urban sites with minimal soil access. Down to Earth 52(2):18-25.

Curtis, A.D. & D.A. Waller. 1997. Problems with the interpretation of mark-release-recapture data in subterranean termites (Isoptera: Rhinotermitidae). Sociobiology 30(3):233-241.

DeMark, J.J., E.P. Benson, P.A. Zungoli & B.M. Kard. 1995. Evaluation of hexaflumuron for termite control in the southeast U.S. Down to Earth 50(1):20-25.

Esenther, G.R. 1980. Estimating the size of subterranean termite colonies by a release-recapture technique. International Research Group on Wood Preservation (1112):1-5.

Evans, T.A. 1997. Evaluation of markers for Australian subterranean termites (Isoptera: Rhinotermitidae & Termitidae). Sociobiology 29(3):277-292.

Evans, T.A., M. Lenz & P.V. Gleeson. 1998. Testing assumptions of mark-recapture protocols for estimating population size using Australian mound-building, subterranean termites. Ecological Entomology 23:139-159.

Forschler, B.T. 1994. Fluorescent Spray Paint as a Topical Marker on Subterranean Termites (Isoptera: Rhinotermitidae). Sociobiology 23(3):27-38.

Forschler, B.T. & J.C. Ryder. 1996. Subterranean termite, *Reticulitermes* spp. (Isoptera: Rhinotermitidae), colony response to baiting with hexaflumuron using a prototype commercial termite baiting system. Journal of Entomological Science 31(1):143-151.

Forschler, B.T. & M.L. Townsend. 1996. Mark-release-recapture estimates of *Reticulitermes* spp. (Isoptera: Rhinotermitidae) colony foraging populations from Georgia, U.S.A. Environmental Entomology 25(5):952-962.

Grace, J.K. & A. Abdallay. 1989. Evaluation of the dye markers Sudan Red 7B with Reticulitermes flavipes (Isoptera: Rhinotermitidae). Sociobiology 15:71-77.

Grace, J.K., A. Abdallay & K.R. Farr. 1989. Eastern subterranean termite (Isoptera: Rhinotermitidae) foraging territories and populations in Toronto. Canadian Entomologist 121:551-556.

Grace, J.K., C.H.M. Tome, T.G. Shelton, R.J. Oshiro & J.R. Yates III. 1996. Baiting studies and considerations with *Coptotermes formosanus* (Isoptera: Rhinotermitidae) in Hawaii. Sociobiology 28(3):511-520.

Haagsma, K. & J. Bean. 1998. Evaluation of a hexaflumuron-based bait to control subterranean termites in southern California (Isoptera: Rhinotermitidae). Sociobiology 31(3):363-369.

Haagsma, K.A. & M.K. Rust. 1995. Colony size estimates, foraging trends, and physiological characteristics of the western subterranean termite (Isoptera: Rhinotermitidae). Environmental Entomology 24(6):1520-1528.

Jones, S.C. 1990a. Colony size of the desert subterranean termite, *Heterotermes aureus* (Isoptera: Rhinotermitidae). Southwestern Naturalist 35:285-291.

Jones, S.C. 1990b. Delineation of *Heterotermes aureus* (Isoptera: Rhinotermitidae) foraging territories in a Sonoran Desert grassland. Environmental Entomology 19(4):1047-1054.

Jones, S.C. 1991. Field evaluation of boron as a bait toxicant for control of *Heterotermes aureus* (Isoptera: Rinotermitidae). Sociobiology 19(1):187-209.

King, J.E. 1999. Laboratory feeding response of eastern subterranean termites (*Reticulitermes flavipes* (Kollar)) to dyed filter paper in no-choice and choice feeding tests. Sociobiology submitted.

Lai, P.Y., J.K. Tamashiro, J.K. Fujii, J.R. Yates & N.-Y. Su. 1983. Sudan red 7B, a dye marker for *Coptotermes formosanus*. Proceedings of the Hawaiian Entomological Society 24:277-282.

Oi, F.M. & N.-Y. Su. 1994. Stains tested for marking *Reticulitermes flavipes* and *R. virginicus* (Isoptera: Rhinotermitidae). Sociobiology 24(3):241-268.

Salih, A. G.M. & J.W.M. Logan. 1990. Histological dyes for marking *Microceratermes lepidus* (Isoptera: Macrotermitinae). Sociobiology 16:247-250.

Su, N.Y. 1994. Field evaluation of a hexaflumuron bait for population suppression of subterranean termites (Isoptera: Rhinotermitidae). Journal of Economic Entomology 87(2):389-397.

Su, N.Y., J.P. LaFage & G.R. Esenther. 1983. Effects of a dye, Sudan Red 7B, on the Formosan subterranean termite. Material und Organismen 18(2):127-133.

Su, N.Y., R.H. Scheffrahn & P. Ban. 1988. Retention time and toxicity of a dye marker, Sudan 7B, on Formosan and Eastern subterranean termites (Isoptera: Rhinotermitidae). Journal of Entomological Science 23(3):235-239.

Su, N.-Y., P. Ban, M. & R.H. Scheffrahn. 1991. Evaluation of twelve dye markers for population studies of the eastern and Formosan subterranean termite (Isoptera: Rhinotermitidae). Sociobiology 19(2):349-362.

Su, N.-Y., P.M. Ban & R.H. Scheffrahn. 1993. Foraging populations and territories of the eastern subterranean termite (Isoptera: Rhinotermitidae) in southeastern Florida. Environmental Entomology 22:1113-1117.

Su, N.-Y. & R.H. Scheffrahn (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.

Su, N.-Y. & R.H. Scheffrahn. 1988. Foraging population and territory of the Formosan subterranean termite (Isoptera: Rhinotermitidae) in an urban environment. Sociobiology 14(2):353-359.

Su, N.-Y. & R.H. Scheffrahn. 1996a. Fate of subterranean termite colonies (Isoptera) after bait applications-an update and review. Sociobiology 27(3):253-275.

Su, N.-Y. & R.H. Scheffrahn. 1996b. A review of the evaluation criteria for bait-toxicant efficacy against field colonies of subterranean termites (Isoptera). Sociobiology 28(3):521-530.

Su, N.-Y., R.H. Scheffrahn & P.M. Ban. 1995a. Effects of sulfluramid-treated bait blocks on field colonies of the Formosan subterranean termite (Isoptera: Rhinotermitidae). Journal of Economic Entomology 88(5):1343-1348.

Su, N.-Y., E.M. Thoms, P.M. Ban & R.H. Scheffrahn. 1995b. Monitoring/baiting station to detect and eliminate foraging populations of subterranean termites (Isoptera: Rhinotermitidae) near structures. Journal of Economic Entomology 88(4):932-936.

Thorne, B.L., E. Russek-Cohen, B.T. Forschler, N.L. Breisch & J.F.A. Traniello. 1996. Evaluation of mark-release-recapture methods for estimating forager population size of subterranean termite (Isoptera: Rhinotermitidae) colonies. Environmental Entomology 25(5):938-951.

Tsunoda, K., H. Matsuoka & T. Yoshimura. 1998. Colony elimination of *Reticulitermes speratus* (Isoptera:Rhinotermitidae) by bait application and the effect on foraging territory. Journal of Economic Entomology 91(6):1383-1986.