Fast Green, a Non-Persistent Dye Marker for Use in Studying Field Populations of Subterranean Termites (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Fast Green, a water-soluble, food grade dye, is suitable for use in the self-mark - capture technique previously developed using Nile Blue and Neutral Red. This was validated for 14 colonies of *Reticulitermes flavipes* at 9 sites in Texas and Oklahoma. In laboratory trials there was no feeding deterrence due to 0.5% Fast Green. Unlike other dyes previously used in field studies, Fast Green does not persist after dyed matrix has been removed. The advantages and disadvantages of persistent and non-persistent dye markers are discussed.

INTRODUCTION

Most previous work on dye markers for subterranean termites has focused on fat soluble dyes which remain in fat bodies of marked termites for long periods after ingestion (Evans 1997, Grace & Abdallay 1989, Grace & Abdallay 1990, Lai *et al.* 1983, Oi 2000, Oi & Su 1994, Su *et al.* 1991). This was necessary to ensure long persistence of marks and to reduce the possibility of transfer of dye among individuals after the initial marking period, both critical criteria for using the mark-release-recapture technique for estimating size of foraging populations.

Recent work (Atkinson 2000) has refined a self-mark / capture method by which foraging subterranean termites feed on a palatable matrix impregnated with marker dyes and are able to acquire a visible mark and spread throughout the foraging territory without active intervention on the part of the researcher. Variations of this technique have been used in several recent studies (Evans 2002, Potter & Hillery 2002, Sajap *et al.* 2000). When using this method, no assumptions are required about the long-term persistence of the mark or its transferability. Given that no estimates of population size are involved, neither criterion of persistence or lack of transferability is important because the proportions of marked and unmarked individuals are not compared. In some cases, lack of persistence could actually be beneficial

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because both Nile Blue A and Neutral Red remain visible in soldiers for periods of up to 1 year after removal of dyed matrix from foraging sites (Atkinson 2000). A less persistent dye marker would allow use of a different, more persistent dye marker if population estimates were desired. Because the dyed matrix is continuously available to foraging termites, the self mark-capture method combines the marking and dispersal phases, rendering questions of transferability moot. In general, for purposes of delimiting foraging territories, transferability is a desirable attribute of potential dye markers.

Oi & Su (1994) discarded several candidate dyes screened against *Reticulitermes flavipes* because they were not persistent, even though they were relatively nontoxic and did leave a visible mark. One of these candidates was Fast Green, a water soluble, food grade dye. Objectives of this study were:

1. Would termites feed on LTC matrix impregnated with 0.5 % (wt./wt.) Fast Green in standard baitube devices?
2. Would termites acquire a visible marking?
3. Would dyed termites move throughout the colony's foraging territory as reflected in the monitoring stations?
4. How long would the dye marker persist after removal of dyed matrix?

MATERIALS AND METHODS

Lab Trials

Fast Green (97%, Aldrich Chemical Co., Milwaukee, WI), Neutral Red (92%, Sigma Chemical Co., St. Louis, MO) and Nile Blue A (87%, Aldrich Chemical Co., Milwaukee, WI) were dissolved in water such that 1.5 ml of solution applied to filter paper circles (Whatman #1, 9.0 cm diameter) would produce concentrations of 0.106, 0.25% 0.50% (wt. / wt.). Dyed paper circles were allowed to dry for 24 hours and then cut into quarter pieces for feeding assays. After drying, quarter-pieces were weighed and folded in half prior to placement in feeding tests.

Eastern subterranean termites (*R. flavipes*) were collected in southern Mississippi and shipped to Dow AgroSciences laboratories in Indianapolis, IN. Termites were maintained on a mixture of wood, corrugated cardboard and moistened filter paper in the laboratory and were used in feeding tests within one month after field collection.

Both force feeding (no-choice) and choice bioassays were run. The test apparatus consisted of two round (5.5 cm diameter) polystyrene containers (harborage and food chambers) connected by tygon tubing (7 cm long, 2.5 mm ID) (King 2000). The harborage chamber contained a 1:1 ratio of vermiculite and white river sand covered by a ventilated

lid. The substrate was moistened with distilled water to the point of saturation before termites were introduced. The food chamber contained the food source(s). One hundred termites were introduced into the harborage chamber. These typically began moving into the food chamber within minutes.

Termite workers were held in the tests for 14-15 days in total darkness at 26° C and 80% RH. At the end of the holding period filter papers were removed, dried and weighed for consumption. Survival and degree of coloration were noted.

Each test (paired treatments) was replicated using different collection sources of *R. flavipes*. For each test, analysis of variance (ANOVA) with protected LSD mean separation test was performed on consumption rates and survivorship. Statistical analyses were computed with Mintab 12.2 for Windows.

Field Trials

Standard baitube™ devices (containing approximately 20 g of matrix) without active ingredient were prepared with Fast Green (.5 % wt. / wt.) using the current Dow AgroSciences LTC commercial matrix.

Dyed matrix was introduced into 14 colonies of *Reticulitermes flavipes* 9 study sites in Texas and Oklahoma. Some of these colonies were at grid sites while others were near residences. Dyed matrix was introduced during 1999-2003, depending on the site. Procedures followed those described by Atkinson (2000). Standard Sentricon® in-ground stations were used at all field sites. At two sites, dyed matrix was introduced inside above ground stations. Stations were checked roughly every 30 days, although this varied according to season and site. Termites were introduced into baitube devices containing the dyed matrix at the time of baiting. In all cases a baitube device containing the green matrix was only introduced into a single station fed on by a particular colony until marked termites appeared at other stations. Other stations with termite activity were either baited with a blank baitube device (matrix with no dye or active ingredient) or with baitube devices containing 0.5% hexaflumuron or 0.5% noviflumuron.

RESULTS

Lab Trials

No-Choice Tests on Fast Green Filter Paper (1999)

No feeding suppression was noted with Fast Green at either 0.1% or 0.5% (Table 1). However, a lower percentage of termites were visibly

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Table 1. No-Choice Test, Forced feeding of eastern subterranean termites to blank and Fast Green dyed filter paper.

Treatment[a]	mg paper consume termite/day d/g ± SEM[b]	% Survival After 15 Days	% Termites Visibly Dyed
Blank Filter Paper	13.36 ± 1.01 a	88.6	0.0
0.1% Fast Green Filter Paper	14.41 ± 1.64 a	88.8	70.3
0.5% Fast Green Filter Paper	13.88 ± 0.72 a	89.4	78.9

[a]All treatments replicated 5 times, 100 termites per replicate.
[b]Means followed by the same letter are not significantly different (ANOVA, $p \geq 0.10$).

dyed with this dye when compared with previous studies on Neutral Red or Nile Blue A (King 2000). Intensity of coloration was highly variable, and there appeared to be less retention of the green dye when compared to the fat soluble dyes. However, there was a lot of green staining in the bioassay units (excreted by termites), this could also serve as a useful marker in the field. No significant mortality was caused Fast Green.

No-Choice Test on Dyed Filter Paper (FP) (2000)

In this test 0.5% Fast Green was compared with 0.1% Nile Blue and 0.25% Neutral Red, with almost all termites becoming visibly dyed (Table 2). Termites consumed Fast Green FP at higher levels than the other dyes, and appeared very green in abdomens only (not fat-soluble). There was very noticeable green staining in the bioassay units from the excrement of termites feeding on Fast Green dye. This effect is not observed to any great degree with the fat-soluble dyes.

Choice Test between Dyed Filter Paper (FP) & Southern Yellow Pine (SYP)

Termites were given the choice to feed between untreated SYP and dyed filter paper (Table 3). Nile Blue A and Neutral Red clearly deterred feeding while feeding on -Fast Green was not different than that on SYP.

Field Trials

Termites which have fed on matrix impregnated with Fast Green acquire a dark green color. Marked termites are clearly distinguishable from undyed individuals. Unlike the case with fat soluble dyes, staining is primarily restricted to the gut line (possibly gut epithelium as well as lumen). Even with fat soluble dyes, however, initial marking after

Table 2. No-Choice Test, Forced feeding of eastern subterranean termites (*R. flavipes*) to blank or dyed filter paper (FP) after 14-day feeding exposure.

Treatment[a]	mg paper consumed/g termite/day (Mean ± SEM)[b]	No. of termite worker survivors/100 after 14 days (Mean ± SEM)[b]	% Surviving termites visibly dyed
Blank FP	21.93 ± 2.50 a	95.25 ± 2.75 a	0.0
0.50% Fast Green FP	20.70 ± 2.07 a	92.50 ± 2.60 a	85.4
0.10% Nile Blue FP	11.01 ± 1.45 c	67.00 ± 14.30 b	100.0
0.25% Neutral Red FP	14.42 ± 2.19 b	88.50 ± 2.90 a	99.7

[a]Each treatment replicated 4 times (100 termite workers per replicate).
[b]Within each column, means followed by the same letter are not significantly different (LSD, p>0.10)

exposure is also along the gut line, gradually passing into abdominal, thoracic and head regions over a period of several days. Termites lightly stained with Fast Green were difficult to distinguish from those lightly marked with Nile Blue A, which typically produces a pale blue coloration under field conditions. Heavily stained individuals were distinguishable. This might limit the utility of Fast Green in close proximity to colonies marked with Nile Blue A. The two are more easily distinguished with practice and if enough dye has been ingested.

Dyed termites were typically found inside the original station by the next visit (Table 4). During most periods of the year, marked termites were also found in other stations at the first visit as well. During winter

Table 3. Choice Test, Comparative consumption of southern yellow pine (SYP) & dyed filter paper (FP) by eastern subterranean termites (*R. flavipes*) after 14-day feeding exposure. Each test replicated 5 times (100 termite workers per replicate).

Paired Choice[a]	mg consumed/g termite/day (Mean ± SEM)[b]	No. of termite worker survivors/100 after 14 days (Mean ± SEM)[c]	% Surviving termites visibly dyed
SYP vs.Blank FP	11.43 ± 2.28[a] 17.94 ± 1.66[a]	87.5 ± 2.18[a]	0.0
SYP vs.0.50% Fast Green FP	15.03 ± 4.31[a] 14.97 ± 2.12[a]	90.0 ± 2.08[a]	68.9
SYP vs.0.10% Nile Blue FP	24.69 ± 1.63[a] 5.47 ± 0.76[b]	82.0 ± 2.55[b]	89.6
SYP vs.0.25% Neutral Red FP	20.26 ± 2.73[a] 10.07 ± 3.06[a]	86.5 ± 2.33[a]	79.7

[a]Each test replicated 5 times (100 termite workers per replicate).
[b]For each paired choice, means followed by the same letter are not significantly different (ANOVA F-test, $p > 0.05$)
[c]Within this column, means followed by the same letter are not significantly different (LSD, p>0.10)

Table 4. Consumption of dyed matrix, movement of dyed termites, and persistence of Fast Green dye marker in colonies of *R. flavipes* in soil stations.

Site	matrix consumed (g)	No. stations with dye	No stations with dyed termites[a]	Max. distance moved (m)	Days after dyed matrix introduced: dyed termites in release station	dyed termites in other stations	all dyed matrix removed	last dyed termites observed
Cuero Garage	32	1 c	7	6.8	31	31	121	121
Austin-Kolb	24	1 c	2	3.1	26	26	63	63
Austin-Williams	37	1	3	15.4	21	21	63	63
Austin-Malloch	16	1	4	16.3	21	21	63	63
Austin-Gingko	36	1	4	12.0	34	34	60	93
Floresville1	10	1	5	11.1	61	61	116	116
Floresville3	17	3	6	7	39	132	132	132
Floresville4	12	1	3	7	45	45	138	138
Woodlands1	64	3	2	7	47	47	159	159
Woodlands2	32	1	2	3	26	26	112	112
Woodlands3	16	1	1	1	47	47	72	72
Woodlands4	62	3	2	1	47	47	101	101
Flower Mound	20	1	2	3	88[b]	88[b]	224	224
Stillwater, OK	45	1	2	3	88[b]	88[b]	187	187

[a] Other than station in which dye was originally released.
[b] First visit subsequent to dye introduction or removal.
[c] Dyed matrix introduced in above-ground stations.

months, this was sometimes slower due to reduced activity and movement of termites under cold conditions.

In all cases there is convincing evidence that the maximum extent of dye movement completely corresponds with the foraging territories as reflected in the station placements, although several colonies tested either had very small territories or had a small area of intersection with the station grid. Other stations at the same sites where green termites did not occur had termites marked with a different dye marker, were widely separated physically, or were not affected when bait matrix containing hexaflumuron or noviflumuron eliminated activity in those stations where green termites had been found previously [Criteria discussed by Atkinson (2000)].

Individuals stained with Fast Green were no longer visible after 30-58 days after the removal of the dyed matrix (Table 4). In almost all cases, no green termites were observed on the next visit following removal of green-dyed matrix. Although Fast Green is not persistent in individuals, it is apparently transferred via trophallaxis because soldiers quickly take on an intense coloration. This confirms previous lab data showing that Fast Green is not persistent in *R. flavipes* (Oi & Su 1994). This short persistence demonstrates that Fast Green only marks termites in the field as long as it is continuously available to the study colony. Multiple release points might be desirable, after connections between these and the original release point have been established.

As noted previously in the lab trials and by (Oi & Su 1994), Fast Green mostly marks the gut line. On several different occasions Fast Green and a different marker dye [Neutral Red and a purple blend (Oi 2000)] were introduced into different stations being visited by the same colony. In these cases individuals marked with both dyes were clearly identifiable. Instead of creating a blended color, the green gut line could be clearly differentiated from the fat body color. In these cases, the green-dyed matrix was removed and the green coloration quickly disappeared from the colony while the fat soluble mark persisted.

DISCUSSION

Nonpersistent dyes offer certain advantages in ecological field studies. In general, the number of different colonies at a site that can be distinguished depends on the number of distinguishable colors. This also assumes that more than one dye is not inadvertently used for the same colony. The effects of dyeing with a nonpersistent dye are rapidly reversible. One problem with persistent dyes is that marked individuals, especially soldiers may retain coloration for up to 1 year. This makes further use of the same dye marker at a site problematic. If what

appear to be 2 separate colonies are marked with different dyes and later turn out to be the same, neither original color or the blend can be used at that same site. If one of the dyes used is nonpersistent, it will clear and can be rapidly refused for a different colony. At long term study sites where in-depth information is desired on a particular colony, Fast Green would be very useful for quickly determining foraging territories. After the dye has cleared, a more persistent, fat soluble dye could then be used for a capture/mark/release/recapture program to obtain an estimate of foraging population size.

In the self-mark technique, the ability to detect marked termites is largely a function of the probability of finding marked individuals. This will depend on the number of foragers in the colony, the persistence of the dye, as well as the rate of consumption of the dyed matrix. With the exception of dye persistence inside termites, Fast Green in practice can be used exactly as Nile Blue A and Neutral Red. Given that empirically, Fast Green seemed to work as well as the more persistent dyes, it may be that the lower persistence is partially offset by the fact that it does not decrease palatability and consumption as do Nile Blue A and Neutral Red.

The dye would be expected to clear after about 30 days during warm weather, but might take longer during colder weather. With Nile Blue or Neutral Red, a single release point is often adequate for stained individuals to appear in widely separated stations and delimit a colony's foraging territory. Because of the lower persistence of Fast Green, multiple release stations should be encouraged, especially those at some distance from the original release point. Although the dye release must begin at a single station, additional stations where dyed individuals are found could be baited with impregnated matrix as well.

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