Lack of Transfer of Permethrin Among Nestmates of *Reticulitermes flavipes* in Laboratory Trials (Isoptera: Rhinotermitidae)[§]

by

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ABSTRACT

The movement of some soil termiticides among subterranean termites from exposed to naïve individuals has raised some interesting questions. Thus far, the only compounds specifically examined for transfer have been termiticides with delayed action, non-repellent active ingredients. We hypothesized that movement of pesticide is possible even for traditional fast-acting repellent termiticides, when applied at concentrations that do not immediately kill the exposed individuals. A simple donor-recipient experiment examining the possibility of transfer of permethrin (as Prelude®) among nestmates of *Reticulitermes flavipes* (Kollar) was performed to test this hypothesis. Results indicated that permethrin is not passed among termites in laboratory settings at the rates used, suggesting that delayed-activity and non-repellence may be necessary traits for the transfer of a termiticide.

Keywords: Permethrin, *Reticulitermes flavipes*, transfer, liquid termiticides, soil barrier, Eastern subterranean termite.

INTRODUCTION

Recent studies have supported the ability of some termiticides, particularly those whose active ingredients are delayed-action, and non-repellent in nature, to affect more termites than just those that contact the material with both drywood and subterranean termite species (Ferster *et al.* 2001, Thorne & Breisch 2001, and Shelton & Grace 2003). While the term 'transfer' has taken hold in the literature for describing this effect, it is very similar to the term 'secondary kill' used more commonly in cockroach management studies *(e.g.,* Appel & Tanley 2000, Buczkowski & Schal 2001). In keeping with the current terminology, transfer will be used in the current paper.

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These studies have employed similar approaches using a simple donor-recipient methodology analogous to some nutrient dynamics studies (Suárez & Thorne 2000) and studies of entomopathogenic fungi (Wright *et al.* 2002). This approach allows researchers to separate mortality within experimental units into that of exposed and naïve individuals with treatment effects normally only measured from the mortality of the naïve (recipient) individuals.

Thus far, only transfer of delayed-action non-repellent active ingredients (as soil termiticides) has been examined (Ferster *et al.* 2001, Thorne & Breisch 2001, Shelton & Grace 2003). This is partially due to the assumptions underlying bait activity studies that these two traits are most important in the acceptance and eventual movement of the material throughout the colony (Esenther & Gray 1968; Su & Scheffrahn 1998), although the argument applies more appropriately to post-digestive metabolites of the bait active ingredients. Since non-repellent soil termiticides have either limited or concentration-dependent repellency depending on the active ingredient involved, it is reasonable to assume that termites encountering treated soil will move through it and return to naïve nestmates, due to delayed toxicological effects. We hypothesized that transfer is possible even for traditional fast-acting termiticides when applied at concentrations that do not immediately kill the donor individuals. This approach does not take into account the repellency of such compounds that would in real world applications likely prevent such transfer from taking place except at very low concentrations. The null hypothesis is that delayed activity contributes to the transfer of soil termiticides. The following study investigates these hypotheses using *Reticulitermes flavipes* (Kollar) termites and the synthetic pyrethroid permethrin – a repellent termiticide (Su & Scheffrahn 1990) with a low LC_{50} for *R. flavipes* in sand (0.68 ppm; Forschler & Townsend 1996).

METHODS AND MATERIALS

Termites were collected from three colonies by taking sawed lengths of termite-infested fallen logs from an undisturbed forest habitat on the John W. Starr Memorial Forest, Mississippi State University, Oktibbeha County, MS. Sections of logs were placed in galvanized steel trash cans (114 L capacity) and returned to the laboratory. Cans were maintained at ambient laboratory conditions (~24°C) and kept closed to retain moisture. Short (~0.5 m x 15 cm) pieces of cardboard were added to the top of each can so that termites could easily forage within them. The cardboard provided a means of extracting small numbers of termites to be used as donors, while entire log sections were extracted to obtain the thousands necessary for recipients. Termites were identified as *R. flavipes* using the key of Hostettler *et al.* (1995). The study used the methods of Shelton & Grace (2003), with some modifications as described.

One week prior to beginning the experiment, a small number (~200-300) of workers and soldiers were obtained from each colony (A, B, and C). These termites were placed into 9.0 cm diameter glass petri dishes lined with two 0.5% Sudan Red 7B (Sigma Aldrich, St. Louis MO) stained filter papers (Whatman International Ltd., Grade #2, 9.0 cm diameter, Maidstone England) and provided with 2 ml of distilled water for moisture. Dishes were kept in an unlit incubator at 25±1°C, ~75% R.H. for 7 d. There was a low level of mortality associated with Sudan Red 7B (as expected, Su *et al.* 1991), however only active individuals were selected as donors.

Treatments were applied using Prelude® (Zeneca Professional Products, Wilmington DE), an EC formulation of permethrin. Distilled water was used to dilute the formulated permethrin, and Silica sand (40-100 mesh, Fisher Scientific Products, Fair Lawn NJ) was used as the application substrate. Concentrations were made in ppm of weight of active ingredient (not formulation) per weight of sand, such that a 6 ml aliquot would treat 25 g of Silica sand (enough for one petri dish). Preliminary results indicated that long-term exposure to permethrin would be fatal to the donors; thus short term exposure (~15 min) at very low concentrations (≤5.0 ppm) were used in exposing donors. For each concentration, 100 g of Silica sand was treated with 24 ml of the appropriate stock solution of permethrin, hand mixed for 1 min in a 0.946 L resealable plastic bag (Hefty One Zip, Pactiv corp., Lake Forest IL), and then emptied onto a disposable aluminum cookie sheet (Hefty EZ foil, Pactiv corp.) to dry for 72 h in a vacuum hood at ambient temperature (~24°C). Once dry, the sand was weighed into plastic disposable petri dishes (Fisherbrand, Fisher Scientific Products, Fair Lawn NJ), at 25 g per dish (15 total, three colonies, five concentrations of permethrin). On the day of the experiment, 6 ml of distilled water was added to each dish for moisture.

Plastic screw top jars (8 x 10 cm) were used as arenas, with each jar representing an experimental unit. Jars were filled with 150 g of Silica sand, and a 2.5 x 3 cm piece of aluminum foil (Reynold's wrap, Reynold's Metal Company, Richmond VA) placed on top of the sand. A single wafer (nominal size 0.5 x 2.5 x 2.0 cm, sanded evenly) of southern yellow pine (*Pinus* Linn. spp.) sapwood was placed on top of the aluminum foil as food for the termites during the test. Finally, 30 ml of distilled water was added to each jar for moisture. Jars were sealed and stored in an unlit incubator (25±1°C, ~75% R.H.) until the test was initiated within 24 h.

A randomized complete block design was used, with five concentrations of permethrin (0, 0.5, 1.0, 2.5, and 5.0 ppm wt a.i./wt sand) and three colonies serving as blocks, and five replicates of each. Thus, there were 75 experimental units in the study. Fresh termites were extracted from each colony; 25 groups of 95 workers (recipients) and one group of ten workers for individual body mass determination were counted. Unstained termites (recipients) were added directly to the jars according to colony affiliation when counted. Groups of 30 stained donor workers were added to appropriate petri dishes according to colony and concentration designation, and allowed to interact with the treated sand for 15 min. Following this treatment, termites were removed and placed in another set of disposable plastic petri dishes each lined with a single clean dry filter paper (Whatman #2). Termites remained on the filter paper for 30 min to dislodge any treated sand grains. Afterward, 5 donors were added to each jar according to concentration and colony affiliation. Jar caps were screwed on loosely, and jars were returned to the incubator to randomly assigned shelves.

After 14 d, the jars were disassembled, and the number of surviving donors and recipients were counted. Survival data for recipients were converted to percentage mortality, arcsine-square root transformed to normalize the data, and subjected to the Mixed procedure in SAS (SAS Institute 1985). The analysis considered colony, concentration, and their interaction as having potential influence on the response (recipient mortality). Colony and its interaction with concentration were defined as random effects in the analysis. The influences of all random effects were tested as part of the Mixed procedure (COVTEST; SAS Institute 1985).

RESULTS

Recipient and donor mortality data are shown in Tables 1 and 2. Individual data sets, when separated by colony and concentration, were not all normally distributed prior to transformation, some having either a left or right skew. For this reason, the data are presented in the tables as medians and first to third quartile ranges. Mean (± SEM) body masses of individuals from each colony were: Colony A 2.13 ± 0.09 mg, Colony B 3.26 ± 0.09 mg, and Colony C 3.06 ± 0.17 mg.

Results from the Mixed procedure indicated that the random effect of colony did not contribute significantly to recipient mortality ($Z = 0.96$; $P = 0.1675$). For the fixed effect of concentration, no significant

Table 1. Median percentage mortality (Q1 - Q3) of recipient termites 14 d after donors were exposed to various concentrations of permethrin (in ppm).

Colony	0 ppm	0.5 ppm	1.0 ppm	2.5 ppm	5.0 ppm
A	30.5(16.3-78.9)	36.8 (32.1-90.5)	24.2(21.6-62.1)	74.7(50.0-91.6)	40.0(34.2-100.0)
B	13.7(11.1-16.8)	13.7 (10.5-19.5)	20.0(13.7-32.1)	35.8(18.9-65.8)	16.8 (11.1-33.2)
C	11.6(10.5-12.6)	8.4 (7.9-10.0)	9.5(6.3-14.7)	10.5(9.5-14.7)	13.7 (10.5-41.1)

Table 2. Median percentage mortality (Q1 - Q3) of donor termites 14 d after exposure to various concentrations of permethrin (in ppm).

Colony	0 ppm	0.5 ppm	1.0 ppm	2.5 ppm	5.0 ppm
A	60 (30-100)	80 (60-100)	40 (20-80)	100 (90-100)	100 (60-100)
B	20 (20-50)	40 (20-50)	20 (10-40)	60 (40-80)	40 (20-50)
C	20 (0-20)	20 (10-20)	20 (10-30)	0 (0-30)	40 (10-50)

differences in recipient mortality were found (F = 2.22; df = 4, 8; P = 0.1560). The influence of the interaction of concentration and colony was estimated to be zero and hence no test was performed by the COVTEST option. It is assumed that the colony * concentration interaction had no significant influence on recipient mortality. Donor mortality data are presented for comparative purposes (Table 2), but were not subjected to statistical analysis.

DISCUSSION

Our hypothesis was that fast-acting compounds may be transferred between donor and recipient termites in a similar manner as non-repellent slow-acting compounds such as the neonicotinoids and pyrazoles. Obviously, the non-repellent nature of these compounds makes them candidates for distribution among termites, because termites are more likely to come into contact with soil treated with a compound that does not repel them. However, our goal was to remove the repellent nature of the compound from consideration by forcing the termites onto treated sand and looking for transfer using faster acting pesticides.

The results of this experiment do not support the hypothesis that permethrin is transferred among nestmates. This information comes with a number of caveats; first the relative numbers of donors to recipients in this study is identical to that used in other studies (5 donors: 95 recipients; Shelton & Grace 2003). It is possible that larger ratios of donors: recipients are necessary for transfer of permethrin. Second, it has been suggested that even the low concentration may have been repellent, thereby reducing social interactions between recipients and donors. While this is a possibility, the mortality associated with further reduction of permethrin concentrations below the LC_{50} (0.68 ppm; Forschler and Townsend 1996), may not have been high enough to produce recipient mortality. Third, the concentrations of permethrin may not have been great enough to induce transfer among the termites in this study. This scenario is unlikely, as preliminary data indicated that the highest concentration used in this study (5 ppm) was high enough to cause donor mortality within a few hours of exposure. However median donor mortality was 100% for only one of the three colonies used in this study at 14 d (Table 2). Higher concentrations, and extended exposure times were unlikely to have any effect other than shortening the lifespan of the donor termites within the test period. For transfer to have wide-reaching effects, *i.e.* killing more than just a few unexposed termites, donors must interact socially with the recipients. Thus a short donor lifespan would be detrimental to the transfer process. Dead donor termites may transfer a limited amount of pesticide to the workers who move the bodies, but such an event is unlikely to cause high percentage mortality among recipients due to the reduced number of termites in direct contact with corpses.

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