Assessment of Donor / Recipient Ratios in Permethrin Transfer Studies with *Reticulitermes virginicus* Banks[1]

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Abstract Crowding of termites may influence observed recipient mortality in donor-recipient toxicant transfer studies. It was hypothesized that if crowding were important, any termiticide (particularly repellent termiticides, which were not thought to transfer) could be transferred by contact among termites when the donor complement was sufficient. Termiticide transfer donor-recipient studies were performed with 5 ppm permethrin (a repellent termiticide; 30 min exposure time) in donor: recipient ratios of 10:90, 20:80, 30:70, 40:60, and 50:50 using 4 colonies of *Reticulitermes virginicus* Banks. Recipient mortality at 14 d was significantly different between treatments and controls in the 30:70 ratio for 3 colonies. Treatments and controls were significantly different for recipient mortality of one colony each in the 40:60 and 50:50 donor: recipient ratios. These results demonstrated that a repellent termiticide could be transferred by contact among termites with high donor percentages; they suggest that the phenomenon of toxicant transfer by contact may be influenced by crowding in these studies.

Key words toxicant transfer, *Reticulitermes*, permethrin, pyrethroids

The movement of termiticides among termite nestmates has been examined frequently over the past decade (Ferster et al. 2001, Thorne and Breisch 2001, Valles and Woodson 2002, Ibrahim et al. 2003, Shelton and Grace 2003). The theory is that pesticides adhering to the integument of termites exposed to the pesticides (donors) are transferred to unexposed nestmates (recipients) through interaction with the donors (Tomalski and Vargo 2004). Haagsma and Rust (2007) in work using termites with sealed mouthparts showed that transfer of imidacloprid was through body contact, not trophallaxis. Toxicant transfer among termites has only been demonstrated in laboratory studies (Hu et al. 2005, Rust and Saran 2006, 2008, Shelton et al. 2006, Song and Hu 2006, Saran and Rust 2007, Bagnères et al. 2009). However, Potter and Hillery (2001) found that foraging at field monitoring stations ceased after a nearby treatment. Work with *Reticulitermes hesperus* Banks (Rust and Saran 2006, Saran and Rust 2007) has shown that due to limits on the amount of pesticide a single donor may carry, mortality only occurs in those termites directly interacting with donors (i.e., recipients are not able to become secondary donors). Also, there are limits on the distance exposed termites can travel, limiting the potential for transfer (Su 2005, Ripa et al. 2007, Quarcoo et al. 2010).

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No traditional repellent termiticides have been shown to transfer by contact among termites in the laboratory (Shelton et al. 2005). However, work with ants (bifenthrin, Soeprono and Rust 2004) and cockroaches (cypermethrin, Schneider and Bennett 1985) has shown that repellent pesticides can be transferred among other insects. Schoknecht et al. (1994) used microencapsulated permethrin as a bait toxicant, which was transferred among termites by trophallaxis. Similarly, Iwata et al. (1989) argued that transmission of microencapsulated fenitrothion among *Coptotermes formosanus* Shiraki individuals was accomplished by grooming. Myles (1996) indicated that termites coated in sulfluramid were groomed by other termites, passing the toxicant through the colony by trophallaxis. Currently, only delayed action, nonrepellent (DANR) termiticides are thought to transfer among termites by bodily contact. Toxicant transfer by bodily contact is likely due to the nonrepellent nature of the compounds, as the speed of lethality varies among various DANR termiticides (Mao et al. 2011).

Various donor ratios have been examined in previous studies (Hu et al. 2005, Song and Hu 2006, Tsunoda 2006, Rust and Saran 2008, Bagnères et al. 2009). Rust and Saran (2008) studied acetamiprid, a DANR termiticide, which was repellent at the doses tested; however, it did transfer among *R. hesperus* using a donor: recipient ratio of 1:1. Acetamiprid is repellent at certain concentrations, and intoxicated insects exhibit delayed effects, a new category (Type III; in the category system of Su et al. 1982, 1987) was proposed for compounds having these characteristics (Rust and Saran 2008). Other researchers have used 1:1 donor: recipient ratios with DANR products, for example, thiamethoxam (Delgarde and Rouland-Lefèvre 2002) and indoxacarb (Hu et al. 2005). Hu et al. (2005) noted an effect of donor ratio and concentration where for 10 ng of indoxacarb/termite (direct application), only the 1:1 donor: recipient ratio had any effect (1:4 and 1:9 were effective for higher concentrations of indoxacarb) against *C. formosanus*. Working with fipronil, Ibrahim et al. (2003) found that using a ratio of fewer than 20% treated *C. formosanus* workers did not cause significant mortality in recipient termites at 24 h post exposure. Their maximum horizontal transmission of fipronil occurred at 40% treated workers (Ibrahim et al. 2003), which approaches the 1:1 ratio (50%) used by Hu et al. (2005) and Rust and Saran (2008).

Donor: recipient ratios in some studies have been kept low (5: 95; Shelton and Grace 2003, Shelton et al. 2006) mainly because it is unlikely that the average termite colony will have enough foragers working on a food source protected by a termiticide that they will be evenly matched with the number of workers feeding elsewhere (a 1:1 ratio). However, this may not always be the case. A small colony with access to a house, but little other coarse woody debris surrounding the structure, may have a majority of the foragers working on the house and, therefore, a larger proportion of the colony exposed directly to the nonrepellent active ingredient. However in that scenario, control would come from direct exposure rather than transfer moving the material among nestmates.

It is possible that donor: recipient ratios can have important implications in transfer studies. Transfer may be a function of simple association among termites, something that happens as a function of crowding, which is a common occurrence in termite assays (Peterson et al. 2004). If transfer is really just a matter of the number of termites having been exposed, then it should be possible to make any toxicant transfer by increasing the number of donors sufficiently. To test this hypothesis, a toxicant that is not known to transfer would be needed. Permethrin is a repellent compound (pyrethroid) that does not transfer under the conditions tested in the literature (using a 5:95 donor: recipient ratio; Shelton et al. 2005).

The mode of action of permethrin is a delay in the closing of voltage-gated sodium channels in nerve cells (Yu 2008). Valles et al. (2000) found a 2-fold difference in LC_{50} between a pair of colonies of *R. flavipes* (Kollar) to permethrin. Permethrin is repellent to termites; either preventing tunneling (Su and Scheffrahn 1990) or causing termites to seal off tunnels (Su et al. 1982) even at low concentrations.

The following study examined permethrin as the insecticide in a simple donor: recipient assay. The study examined 10:90, 20:80, 30:70, 40:60, and 50:50 donors: recipients in small jar arenas. Recipient mortality was compared among treatments and controls (within colonies) for each donor: recipient category. The null hypothesis was that no transfer happens (i.e., recipient mortality is equivalent to controls) with any donor: recipient ratio tested when using permethrin as the active ingredient. The alternative hypothesis was that permethrin is transferred (recipient mortality greater than controls) in at least one of the donor: recipient ratios tested.

Materials and Methods

Termites. Termites were collected from infested lumber obtained from the John W. Starr Forest, maintained by Mississippi State University (Oktibbeha Co., MS). Infested lumber was sectioned into 0.3 - 0.5 m lengths and stored in 114 L metal trash cans in the laboratory. Termites were extracted from the lumber immediately before use in studies. Termites were identified as *Reticulitermes virginicus* Banks using soldier morphology characters as described by Hostettler et al. (1995).

Experimental design. This study was a simple donor: recipient experiment using methods similar to those in the literature (Thorne and Breisch 2001, Ferster et al. 2001, Shelton and Grace 2003). These studies use stained, treated termites (donors) mixed with unstained, unexposed nestmates (recipients), and examined mortality in the recipient population after 14 d. Donors were treated by allowing the termites to walk on treated sand for a period of time. This study used a single concentration of permethrin (5 ppm) and a 30-min donor exposure period. There were 5 donor: recipient treatments (10: 90, 20: 80, 30: 70, 40: 60, and 50: 50 permethrin-treated donors: unexposed recipients; 100 workers total per replicate) with matching controls (10: 90, 20: 80, 30: 70, 40: 60, and 50: 50 water-treated donors: unexposed recipients). There were 5 replicates of each treatment and control group per colony. Four colonies (A-D) of *R. virginicus* were used in this study. These studies were performed over a series of 5 wks (one per donor: recipient ratio) within 4 months of the collection of the termite colonies from the field. Experiments with colonies A-C were performed concurrently, and colony D experiments were performed at a later date.

Staining. Groups of ~200 workers were collected from each colony and placed on 2 sheets of 0.25% Sudan Red 7B stained filter paper (Whatman #2; 9.0 cm diam, Whatman International, Ltd.) each moistened with 1 ml distilled water in glass Petri dishes. All Petri dishes were then placed into a 25 ± 1°C incubator at >70% R.H. Termites were allowed to feed on the stained filter paper for 7 d prior to the treatment day of the study. These termites were the exposed (donor) termites used in the study.

Termiticide and substrate preparation. This study used 5 ppm permethrin treated sand as an exposure substrate and an exposure time of 30 min. Preliminary studies with colony B showed that higher concentrations and longer time periods led to unacceptably short survival times for donors. Concentration and exposure times were held constant because the variable of interest is the ratio of donors: recipients in the study. An EC formulation of permethrin, Prelude® (Zeneca Professional Products,

Wilmington DE) was used, and all dilutions made using distilled water. A stock solution was made such that a 6 ml aliquot would treat 25 g of sand resulting in 5 ppm (wt. a.i. per wt. sand). To ensure that donors were treated equally, a large number of dishes of treated sand were used each treating only 60 termites. Each Petri dish of treated (permethrin or control) sand was used only once, with fresh control and permethrin treated sand prepared each week. To treat the sand, 100 g aliquots of sand (Fisherbrand, Thermo Fisher Scientific, Pittsburgh, PA) were treated with 24 ml of the stock solution separately in one quart plastic sealable bags. The sand was then hand mixed for 1 min, and emptied into a 20 × 20 cm aluminum pan (one per aliquot) to dry under a hood for 96 h. For the controls, a separate set of 100 g aliquots of sand were treated with distilled water. Control sand was mixed and dried as described above for the permethrin treated sand. On the day before treatment, plastic Petri dishes (100 × 20 mm; Fisherbrand, Thermo Fisher Scientific, Pittsburgh, PA) were filled with 25 g of either permethrin-treated or water-treated sand.

Arenas. Arenas consisted of 8 cm × 10 cm screw top plastic jars (473 ml, Qorpak jars, Cole Parmer, Vernon Hills, IL), each containing 150 g of sand moistened with 27 ml of distilled water on the day before treatment day. A 2.5 × 3 cm piece of aluminum foil was placed in the center of the jar on top of the sand with a wafer of southern yellow pine (*Pinus* spp. L.; 0.5 × 2.5 × 2.0 cm) on top. Once the sand was moistened, all jars were closed loosely and placed in an incubator at 25 ± 1°C, >70% RH.

Treatment. On the day the experiment began (treatment day), ~1000 termites were collected from each colony. Each Petri dish of treated sand (both permethrin and control) was moistened with 5 ml of distilled water, and set aside until the treatment procedure. Termites were counted into groups of 90, 80, 70, 60, or 50 termites each (5 per treatment per colony), depending on the donor: recipient ratio being tested. The unstained termites were the recipients in the test, and each jar was provided with a group of the appropriate colony recipients.

Stained termites were counted into groups of 60 workers and 1 group placed on each Petri dish of treated and untreated sand. After 30 min the termites were removed and placed on clean dry filter papers in a second set of plastic Petri dishes, where they were allowed to walk for 30 min (providing time to remove any sand particles adhering to the termites). Finally, the donors were counted into groups of appropriate donor number for each jar, and the donors added to the appropriate jars. All jars were then loosely closed and returned to the incubator for 14 d. At the end of the 14 d, all jars were dismantled and the surviving donors (stained) and recipient (unstained) termites counted and recorded.

Analysis. Analyses were performed on recipient mortality calculated from the surviving recipient termites in each jar. Percentage donor and recipient mortalities were subjected (separately) to Kruskal-Wallis procedure using SAS ($\alpha = 0.05$; proc npar1way; SAS Institute 1985). Comparisons were made within donor: recipient ratio and within colony.

Results

Median percentage donor mortality is illustrated in Fig. 1. Kruskal-Wallis comparisons of median donor mortality within colony and donor: recipient ratio indicated that not all of the donor mortalities were significantly different from the controls (Fig. 1). For all ratios tested, there was less mortality among permethrin-treated donors from colony D than treated donors from the other colonies.

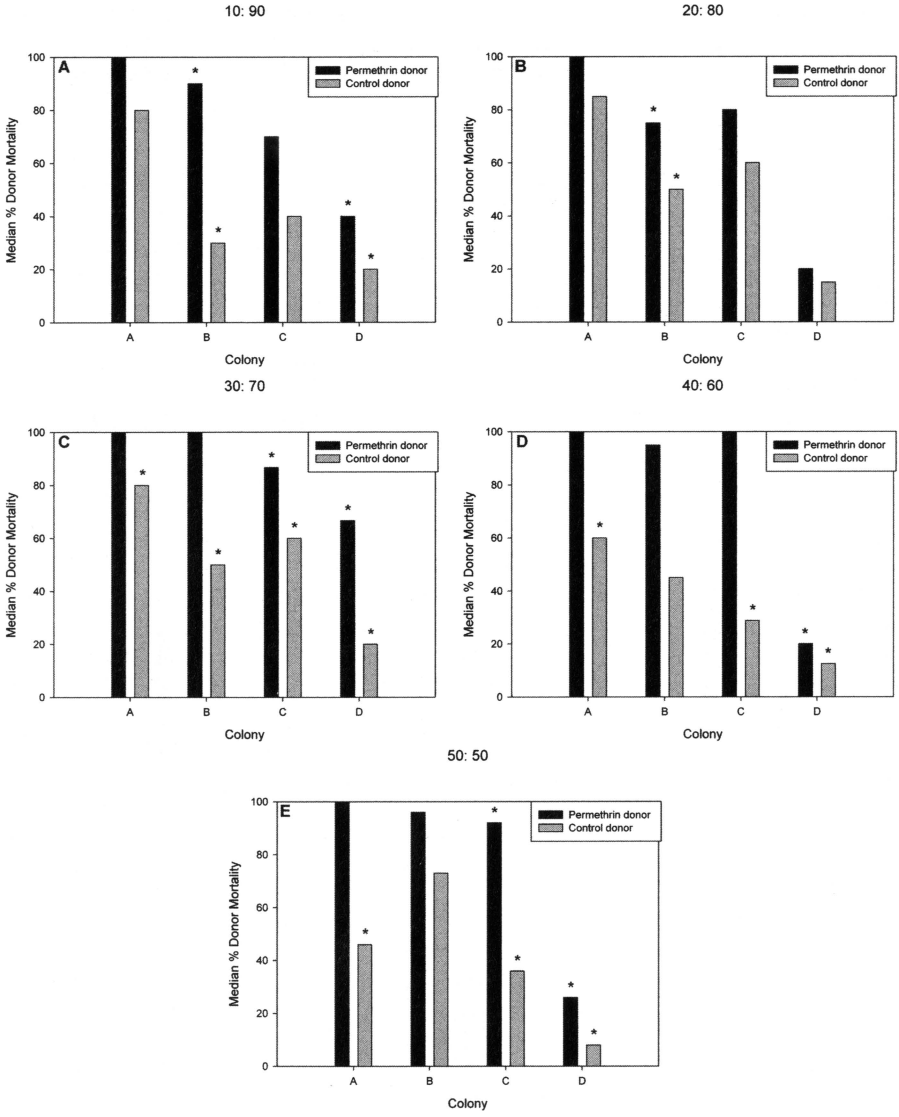

Fig. 1. Median percentage mortality of donor *R. virginicus* workers at various donor: recipient ratios at 14 d. Donors were exposed to 5 ppm permethrin for 30 min. Donor: recipient ratios were 10:90 for graph A, 20:80 for B, 30:70 for C, 40:60 for D, and 50:50 for E. Asterisks indicate a significant difference between controls and treatments within colony and within donor: recipient ratio.

Median percentage recipient mortality is illustrated in Fig. 2. Statistics for the comparisons are provided in Table 1. Percentage recipient mortality was not normally distributed, therefore nonparametric analysis was used. From Fig. 2 it can be seen

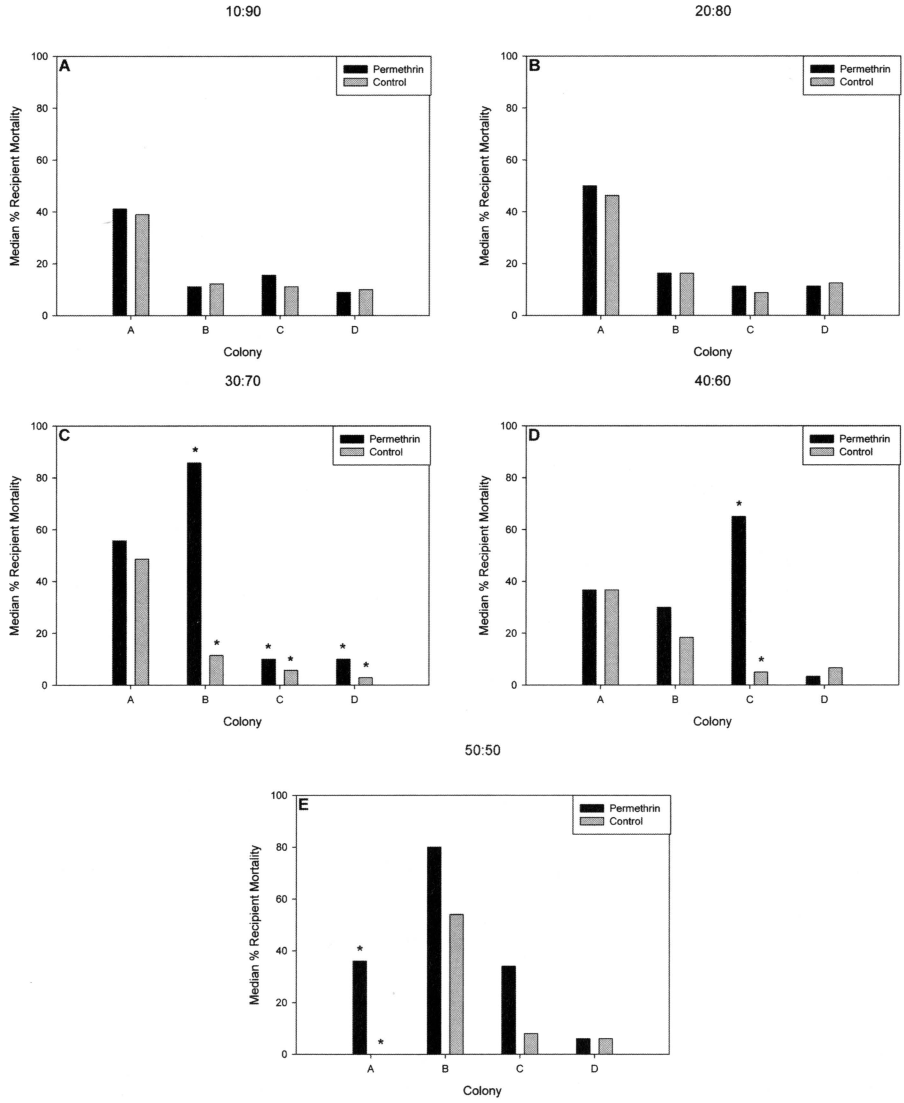

Fig. 2. Median percentage mortality of recipient *R. virginicus* workers at various donor: recipient ratios at 14 d. Donors were exposed to 5 ppm permethrin for 30 min. Donor: recipient ratios were 10:90 for graph A, 20:80 for B, 30:70 for C, 40:60 for D, and 50:50 for E. Asterisks indicate a significant difference between controls and treatments within colony and within donor: recipient ratio.

Table 1. Results of Kruskal-Wallis procedure on percentage mortality of recipient *R. virginicus* workers at various donor: recipient ratios at 14 d (comparisons within donor: recipient ratio and within colony).

Donor: Recipient Ratio	Colony	Chi-square	P
10:90	A	0.0000	1.0000
	B	0.1006	0.7511
	C	1.1043	0.2933
	D	0.1019	0.7496
20:80	A	1.3200	0.2506
	B	0.5478	0.4592
	C	0.8945	0.3443
	D	0.7067	0.4005
30:70	A	0.0982	0.7540
	B	6.8598	*0.0088*
	C	3.9865	*0.0459*
	D	4.4444	*0.0350*
40:60	A	0.1767	0.6742
	B	1.5805	0.2087
	C	6.0000	*0.0143*
	D	0.0468	0.8288
50:50	A	6.4800	*0.0109*
	B	1.2356	0.2663
	C	1.3362	0.2477
	D	0.1019	0.7496

that in both the 10:90 and 20:80 donor: recipient ratios, permethrin treated donors did not significantly increase recipient mortality above the level of the controls for any colony. For the 30:70 donor: recipient ratio, colonies B, C, and D all had a significant difference between jars with water-treated *vs.* permethrin-treated donors. There was also a significant difference between the treatment and control groups for the 40:60 donor: recipient ratio for colony C. Finally, a significant difference was found between the treatment and control groups for colony A in the 50:50 donor: recipient ratio.

Discussion

Donor mortality data indicated that colony D was less susceptible to permethrin at 5 ppm and 30 min exposure (Fig. 1). This is also apparent in the recipient mortality in Fig. 2; obviously it is unlikely that high levels of recipient mortality will be found with

colony D when the donor mortality is so low. Whereas it is interesting that the colonies selected for this study varied in their vigor and susceptibility to permethrin, colony D termites may not be able to provide information regarding transfer due to their low susceptibility (Fig. 1). Donor control mortality was very high (Fig. 1), indicating an influence of the stain or perhaps the extra handling that the staining required.

Recipient mortality data indicate that movement of permethrin occurs with 30:70 donor: recipient ratio under these conditions, and can occur at higher ratios as well dependent on colony. However, the median recipient treatment mortalities for colonies C and D at the 30:70 donor: recipient ratio are low, and may not be biologically meaningful. These results are similar to other results in the literature. Song and Hu (2006) demonstrated that increasing the donor: recipient ratio reduced the amount of time to reach 90 - 100% mortality in *C. formosanus* workers with donors exposed to fipronil. Hu et al. (2005) showed that increasing the concentration of donor exposure to indoxacarb reduced the donor: recipient ratio necessary for significant mortality in *C. formosanus* recipients.

Recipient mortality for some colonies, whereas significantly different from the controls, was not of a level that would be associated with control of a population (particularly colonies C and D at 30:70 donor: recipient ratio). Low recipient mortality has occurred in other transfer studies [Shelton and Grace 2003 (fipronil and imidacloprid), Saran and Rust 2007 (fipronil), Rust and Saran 2008 (acetamiprid)]. Whereas colony differences have been observed (Shelton and Grace 2003) in transfer studies, these are more likely due to variability in susceptibility to the insecticides (Osbrink et al. 2001) than due to behavioral differences among the colonies (Shelton 2009).

These results indicate that in contrast to previous studies (Shelton et al. 2005) permethrin is capable of being passed by contact among workers of *R. virginicus* in laboratory trials. The previous work with *R. flavipes* (Kollar) used a 5:95 donor: recipient ratio and a shorter exposure time (15 min *vs.* 30 min in the current study; Shelton et al. 2005). In the current study transfer was not noticed in ratios lower than 30:70 donors: recipients, so it is not surprising that the previous study showed a lack of transfer with permethrin.

These data indicate that a pyrethroid termiticide is capable of being passed by contact among termite workers under laboratory conditions, a characteristic previously thought to only apply to DANR compounds. This suggests that transfer of materials among workers is a basic component of termite social interactions and may apply to a number of other products (pheromones, fungal spores, etc.; Wright et al. 2002) aside from termiticides. As in most studies of this type, the exact interactions between donors and recipients were not known (Quarcoo et al. 2010). The interactions may have been as simple as recipients grooming the donors, or as involved as cannibalism of donor corpses. Regardless of the route of toxicant uptake, if both repellent and DANR compounds are able to be transferred (even at large ratios of donors to recipients) then the same control expectations should be made for both groups of compounds. It is unlikely that termites will penetrate soil treated with a repellent termiticide, reducing the possibility of permethrin being transferred among termites in the field. The difference in transfer between the 2 groups of compounds appears to be in the number of donors exposed to the pesticide, with permethrin requiring as much as a 30: 70 donor: recipient ratio for transfer. This has been demonstrated in acetamiprid, a DANR pesticide that is repellent at certain doses (Rust and Saran 2008).

The requirement of high donor: recipient ratios for transfer of permethrin may be explained by the fact that recipient termites are confined to a small volume of sand

(in jars) with a large number of exposed donors. After a certain ratio of donors: recipients, it may no longer be possible for recipient termites to avoid contact with the donors. This assumes that termites are able to discriminate between donors and other nestmates, which may be possible when donors are exposed to a repellent compound like permethrin.

Of the 4 colonies, it is apparent that colony A was a low vigor colony (Lenz 2005, Arquette et al. 2006, Arquette and Forschler 2006) given the high recipient control mortality in all but the 50:50 donor: recipient ratio trial. Colony A was included to demonstrate the problems with low vigor colonies in laboratory studies. Although all colonies were used within 4 months of initial capture, in the 50:50 donor: recipient comparison (the final week of the study), colony B appears to be reduced in vigor as well (Fig. 2). In the 50:50 donor: recipient comparison, colony A appears to be of comparable vigor to colonies C and D. Colonies C and D were apparently healthy throughout the experiments (Fig. 2). This variability in response observed in colonies A and B is one of the problems in working with colonies in suboptimal condition. Even between the 2 consistently healthy colonies (C and D), variability in response exists for this transfer effect. Whereas both colonies indicated a significant response at the 30:70 donor: recipient ratio, only colony C had a significant response at the 40:60 donor: recipient ratio (Table 1; Fig. 1).

This study has shown that permethrin (a repellent termiticide) can be passed among individuals of *R. virginicus* when sufficient donors are exposed to the material. Whereas it is possible for permethrin to be passed among individuals, the colony origin of those individuals can influence the results. Colony vigor can introduce variability in the results. These data indicate that the number of possible compounds that can transfer is larger than previously understood.

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