Effects of Dose, Donor-Recipient Interaction Time and Ratio on Fipronil Transmission Among the Formosan Subterranean Termite Nestmates (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Transfer of fipronil's lethal effect was studied against the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. Donor workers were topically dosed with 2 different doses and placed together with recipient workers at 3 different donor-recipient ratios to observe recipient mortality at designed intervals for 20 days. Recipients exposed to donors treated at10 ng a.i./donor yielded full expression of 90-100% mortality within 4 and 12 days at ratio of 1:1 and ≤ 2:8, respectively. Recipients exposed to donors treated at 2 ng a.i./donor only showed < 48, 21, and 15.6% mortality at ratio of 1:1, 2:8, and 1:9, respectively, and the full expression of mortality appeared within 16 days. These results support the hypothesis that the time required for a full expression of transferable lethal effects of fipronil in untreated termites increases as the dose on treated termites decreases at given donor-recipient ratios. Recipients exposed to donors treated with a high dose and at high donor-recipient ratio show greater mortality compared to those exposed to donors treated with a low dose and at low donor-recipient ratio. Recipient mortality increases as the donor-recipient interaction time increases until full expression of transferred lethal effect. Donors had greater mortality than recipients at a given observation date before both donors and recipients reached 100% mortality.

Keywords: *Coptotermes formosanus*, non-repellent termiticide, toxic transmission, social insects, termite control.

INTRODUCTION

Subterranean termites are social insects living in colonies that form networks underground and above ground. Subterranean termites are wood-feeders

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and can cause serious economic damage to buildings and agricultural plants. Among them, the introduced Formosan subterranean termites, *Coptotermes formosanus* Shiraki, is the most damaging species (Su & Scheffrahn 1988). Due to the Formosan's extraordinary economic importance, intensive research has been being centered on how to effectively control termites. Currently, insecticides used for termite control can be categorized into three groups: organochlorine cyclodienes, which are being phased out of use because of potential damage to the environment and human health; pyrethroids, which are considered not effective because of their repellency and short residual life in soil; and new termiticides that are considered as nonrepellent with a delayed mode of action (Potter & Hillery 2003). Besides the new nonrepellent and slow-acting termiticides, many new technologies and products have also been developed in recent years, including baiting systems, insecticide-impregnated plastic barriers, and various physical barriers. However, soil treatment with termiticides remains popular in termite control (Anonymous 2002, Su 2002, Su *et al.* 2004).

One of the new termiticides is Fipronil (Termidor®), a phenyl pyrazole chemical that interferes with the function of the central nervous system. It has been demonstrated in laboratory studies that fipronil does not repel termites from either tunneling into treated soil or remaining in contact with the treated soil long enough to acquire lethal doses (Hu 2005). Fipronil has also been reported to have a delayed action that allows the contaminated termites to maintain normal behaviors for an extended period so as to be able to transfer its lethal effects to those unexposed colony mates through social interactions and thus cause secondary mortality in termites (Osbrink & Lax 2002, Ibrahim *et al.* 2003, Shleton & Grace 2003, Remmen & Su 2005a). Field studies have shown that the use of fipronil has a greater impact on termite populations than organochlorine cyclodienes and pyrethroids and can provide 100% control for more than 10 years (Hu & Hickman 2006 in press, Wagner *et al.* 2005). It is suggested that fipronil's success is partly due to the transferability of its lethal effects, which is considered important in controlling pest species of social insects such as termites (Thorne & Berisch 2001).

Previous studies used doses or concentrations greater than LD_{50} or LT_{50} for fipronil's translatability in *C. formosanus*, and evaluated its transfer effects within relatively short periods of less than 72 h (Shelton & Grace 2003, Ibra-

him *et al.* 2003). Recent studies reported that the time for fipronil to lead to 50% mortality (LT_{50}) in termite workers can be concentration-dependent or dose-dependent (Remmen & Su 2005a, Remmen & Su 2005b). This study was designed to investigate how doses (lower and greater than reported LD_{50} by Ibrahim *et al.* 2003), donor-recipient interaction time (up to 20 days), and donor-recipient ratio (1:1, 2:8, and 1:9) would affect the full expressions of transferable lethal effects of fipronil in *C. formosanus.* Our hypothesis was that the times required for a full expression of transferable lethal effects in untreated termites might increase as the doses on treated termites decrease at given donor-recipient ratios.

MATERIALS AND METHODS

Groups of *C. formosanus* were collected from two field colonies in Opelika, Alabama, using underground open-bottom traps described by Hu & Appel (2004). A trap was comprised of a plastic bucket (18 cm in height by 13 cm in diameter) and a cardboard roll (15 cm in height by 11 cm diameter). Termites in cardboard rolls were held in covered plastic boxes (13.5 × 19 × 13 cm) in an incubator at 22 ± 1 °C and 90 ± 3% RH for < 2 days before use.

Experimental protocol was similar to that described by Hu *et al.* (2005). Seven days prior to beginning the experiment, termites from each colony were gently tapped out of cardboard rolls and divided into two groups. One of the two groups was placed into 150-mm diameter Petri dishes lined with 2 filter papers (Whatman International Ltd., 9.00-cm diameter, Maidstone, England) stained with 6-ml 0.01% aqueous solution of Nile blue A (Aldrich, Milwaukee, WI). Termites fed on the stained filter paper did not express abnormal behavior or mortality (Hu & Zhu 2003). The other group was placed into similar Petri dishes lined with plain filter papers moistened with 6-ml distilled water. To ascertain that termite mortality would be solely caused by fipronil intoxication, only active intact workers (fourth instar or older) were used for testing. Fipronil (Termidor®, BASF Corp., Research Triangle Park, NC) was diluted with distilled water to obtain 0.001 or 0.005 % a.i. concentration, so that when 0.2-µl droplet of 0.001 or 0.005% a.i. was applied to a termite, a dose of 2 or 10 ng per termite would be achieved.

A revised donor-recipient bioassay model by Hu *et al.* (2005) was used in this study. First, a stained worker was held still with a forceps in a glass Petri

dish. A 0.2-µl droplet of a solution was topically applied on its dorsal thorax using a 1-µl micro-syringe (Hamilton, Reno, NV). The volume of 0.2 µl provided a full coverage of the thorax dorsum without runoff (Hu *et al.* 2005). In cases where a droplet was misplaced or ran off, the treated termite was excluded from testing. Distilled water was used as the control. The topically dosed termites (hereafter referred to as donors) were left in separate glass Petri dishes until the droplet dried off. Donors and unstained untreated workers (hereafter referred to as recipients) from the same colony were introduced into 6-cm diameter Petri dishes to obtain three donor-recipient ratios of 1:1, 1:4, and 1:9 in groups of 100 workers. Each dish contained 10-g sterilized sand moistened with 1.5-ml distilled water and a moistened spruce wood block (3 × 2 × 0.5 cm) wrapped with a piece of Whatman No. 1 filter paper (6 by 4 cm) serving as a food source. Petri dishes were held in a covered plastic box (13.5 × 19 ×11 cm) and maintained in an incubator at 22 ± 1 °C in total darkness except during observation. Mortalities of recipients and donors were recorded at 2-day intervals through 4 days followed by 4-day intervals until the full expression of the lethal effect was observed by counting the number of survivors. Termites were considered dead when they no longer responded to probing with forceps.

Termite survival data for recipients were pooled and converted to percentage mortality because previous test had shown no significant difference of fipronil susceptibility between the two colonies (Hu 2005). Percentage of mortality was corrected using the formula of Abbott (1925), normalized using arcsine square-root transformation, and subjected to general analysis of variance (ANOVA) to evaluate what effects dose, donor-recipient interaction time (days) and donor-recipient ratio had on recipient mortality. Significant differences ($\alpha = 0.05$) were separated for each ratio using Tukey comparison procedure (Statistix® 8, 2003). The mean percentages of actual mortality were reported in figures and table.

RESULTS

The transfer of fipronil's lethal effect was indicated by recipient mortality (Figs. 1, 2 and 3). Dose had significant effect on lethal transfer ($F_{(71,11)} \geq 18.6$, $P < 0.05$). At any given donor-recipient interaction date, the higher dose of 10 ng/donor always had significantly greater recipient mortality than the

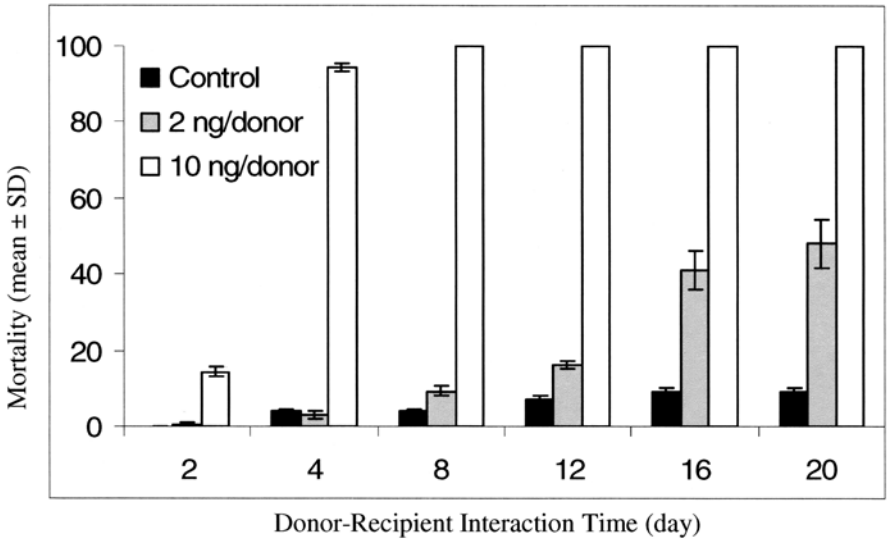

Fig. 1. Mortality of recipient termites after mixing with donors topically dosed with fipronil at donor-recipient ratio 1:1 in groups of 100 *C. formosanus* workers

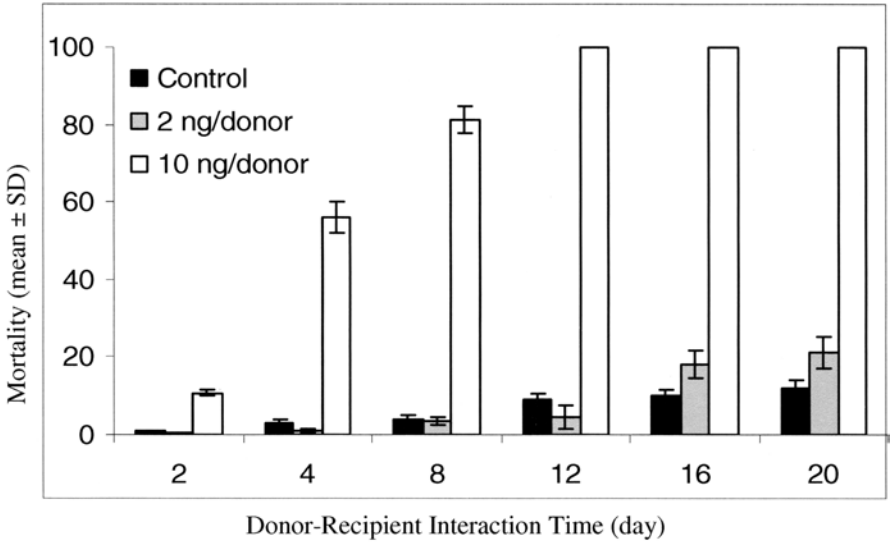

Fig. 2. Mortality of recipient termites after mixing with donors topically dosed with fipronil at donor-recipient ratio 2:8 in groups of 100 *C. formosanus* workers

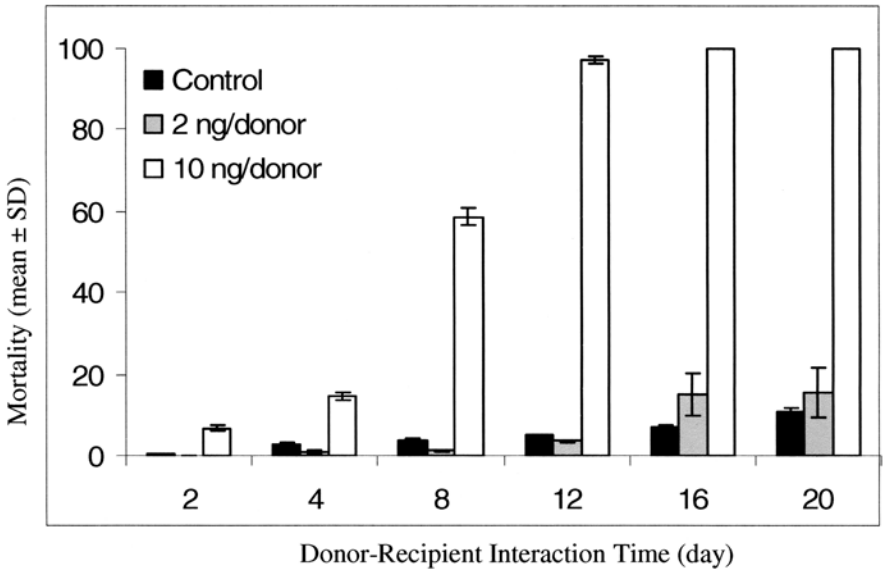

Fig. 3. Mortality of recipient termites after mixing with donors topically dosed with fipronil at donor-recipient ratio 1:9 in groups of 100 *C. formosanus* workers

lower dose of 2 ng/donor. When the study was terminated at 20 days, the higher dose killed 100% of the recipients independent of ratios, while the lower dose killed only 48, 21, and 15.6% recipients at ratios of 1:1, 2:8, and 1:9, respectively.

Recipient mortality was also significantly affected by donor-recipient interaction time ($F_{(35,5)} \geq 44.7$, $P < 0.05$). The donor-recipient interaction time required to show full expression of adverse effects on recipients was dose-dependent. Full expression of transferred lethal effect was when recipient mortality would not significantly increase as the time increased. The low dose of 2 ng/donor took considerably longer to fully express the transferred lethal effect than the high dose of 10 ng/donor. It took as long as 16 days for the dose of 2 ng/donor to fully express its transferable lethal effect at the tested ratios. However, it only took 4 or 12 days for the dose of 10 ng/donor to kill 90-100% recipients at ratio of 1:1 or ≤ 2:8.

The direct toxicity of fipronil was indicated by donor mortality (Table 1). As expected, donor mortality was consistently greater than recipient mortality at any given day before both donors and recipients reached 100% mortality.

DISCUSSION

The results of this study support the hypothesis that the time required for a full expression of transferred fipronil increases as the dose delivered on donor decreases, in a similar manner (but maybe different pattern and level) to the expression of LT_{50} and LD_{50} in termites directly treated with fipronil. High dose and high donor-recipient ratio can cause greater mortality in a relatively shorter time than low dose and ratio. When exposed to donors with high dose at high donor-recipient ratios, recipients can acquire more of the fipronil from donors and have more chances to interact with donors, thus quickly acquiring fatal doses (Ibrahim *et al.* 2003, Hu *et al.* 2005). A threshold of 2.5 ng fipronil per donor was reported by Ibrahim *et al.* (2003) to cause >40% recipient mortality within 1, 2, or 3 days at donor-recipient ratio of 3:7, 2:8 or 1:9, respectively. In our study, the dose of 2 ng per donor resulted in 41-48% recipient mortality within 16 days at ratio of 1:1, indicating that the transferable threshold of fipronil can be time-, and ratio-dependent. At the low dose, we observed no significant increase in mortality of either donors or recipients after 16 days regardless of the donor-recipient ratios (Table 1, Figs. 1, 2, and 3). It is possible that at such low dose, topically applied fipronil was not available for transfer anymore after 16 days under the conditions of the experiment.

Slow-acting and nonrepellency are considered important transferable properties of a termiticide (Thorne & Breisch 2001). Fast-killing and repellent insecticides such as pyrethroids are not transferable (Shelton *et al.* 2005). Donors who are directly exposed to fipronil

Table 1. Mortality of donor termites (mean% ± SE) after mixing with recipient termites in groups of 100 *C. formosanus* workers

Interacting day	Donor-recipient ratio 50:50			Donor-recipient ratio 20:80			Donor-recipient ratio 10:90		
	Dose of fipronil (ng a.i./donor)								
	0	2	10	0	2	10	0	2	10
2	0 ± 0	0.7 ± 0.4	30.9 ± 3.4	0.8 ± 0.1	1.2 ± 0.8	25.2 ± 3.1	0.5 ± 0.2	1.7 ± 0.3	13.3 ± 2.1
4	2 ± 0.3	2.0 ± 0.5	98.1 ± 0.6	3 ± 0.2	1.7 ± 1.1	89.6 ± 3.8	3 ± 0.6	3.3 ± 0.8	56.7 ± 4.2
8	4 ± 0.4	15.6 ± 1.5	100 ± 0	4 ± 0.4	14.1 ± 2.9	98.6 ± 0.5	4 ± 0.4	8.3 ± 1.6	95.3 ± 3.6
12	7 ± 0.4	29.5 ± 1.7	100 ± 0	9 ± 0.5	24.5 ± 1.4	100 ± 0	5 ± 0.5	18.4 ± 2.2	100 ± 0
16	9 ± 0.8	56.9 ± 2.3	100 ± 0	10 ± 0.4	52 ± 3.1	100 ± 0	7 ± 0.4	39.8 ± 3.1	100 ± 0
20	9 ± 0.8	62.6 ± 3.1	100 ± 0	12 ± 0.8	53.4 ± 3.3	100 ± 0	11 ± 0.2	42.3 ± 2.4	100 ± 0

are expected to exhibit greater mortality than recipients at a given dose, donor-recipient ratio, and observation date. On the other hand, recipients who have to acquire fipronil through social interactions from donors, intoxicated recipients (also called secondary donors), or possibly casual contact with contaminated substrate in the test arena are expected to have delayed mortality that may be lower than that of donors (Hu *et al.* 2005). Several transfer mechanisms have been proposed and some of them are observed (Hu *et al.* 2005, Tomalski & Vargo 2005). The most common mechanisms observed are contacting, grooming, trophallaxis, and/or cannibalism/necrophagy between live exposed and unexposed termites. Unexposed termites can also acquire the toxicant from dead exposed termites by moving or cannibalizing the dead bodies (Hu & Song, unpublished).

Together with previous reports, this study corroborates the statement by Thorne and Breisch (2001) that using nonrepellent slow-acting insecticides for termite control can provide population suppression by eliminating groups, populations, and eventually colonies through direct contact or cross-transfer effects. This study provides a rationalization for field discovery of dead/dying termites in independent in-ground monitors outside treated zone and the success of using fipronil application as an IPM option (Hu & Hickman, in press). Indeed, a successful long-term termite management should involve multiple tactics addressing landscape and construction features implicating termite problems, surveillance, inspections, education, training, and collaboration of all termite-affected parties. The use of fipronil is a rational termiticide application that takes advantage of lethal effect transfer to achieve population suppression at colony level.

ACKNOWLEDGMENTS

We thank Shizhu Li (Auburn University) for termite collection, Dr. Arthur Appel and Jamie Creamer (Auburn University) for critical review to improve earlier versions of this manuscript.

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