Performance of Termidor® HE High-Efficiency Termiticide Co-pack with Termidor® HE Technology Against Eastern Subterranean Termites *Reticulitermes flavipes* and Formosan Subterranean Termite *Coptotermes formosanus* in Laboratory Trials and Field Applications

by

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ABSTRACT

Two laboratory and one field study were performed utilizing fipronil ($C_{12}H_4Cl_{l2}F_6N_4OS$), in the form of Termidor® HE Co-pack with Termidor® HE Technology. Laboratory studies included glass tube bioassays and collateral transfer effect trials. Glass tube bioassay results indicated that this formulation of Termidor® HE Co-pack was efficacious against *Reticulitermes flavipes* and *Coptotermes formosanus* caused 100% mortality of both species by 72 h post-exposure. Collateral transfer effect trials also showed efficacy of Termidor® HE Co-Pack with Termidor® HE Technology against both *R. flavipes* and *C. formosanus* by causing 100% mortality by 96 h. Termidor® HE Co-pack with Termidor® HE Technology was also applied to structures as a post-construction treatment for remediation of subterranean termite infestations. In field trials, 12 termite structures were treated with Termidor® HE Co-pack with Termidor® HE Technology (hereafter referred to as Termidor® HE) and no subterranean termites were detected through 36 months post-treatment.

Key Words: Termidor® HE Co-pack, *Reticulitermes flavipes*, *Coptotermes formosanus*

INTRODUCTION

Fipronil ($C_{12}H_4Cl_2F_6N_4OS$) is a phenylpyrazole (Tingle et al. 2003) insec-

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ticide first discovered in 1987 by Rhone-Poluenc Agro (Tomlin 2000). It was first introduced and registered in the United States in 1996 (Bobe et al. 1998, Ware 2000) for use against piercing, sucking chewing insect pests (Bostian et al.1996). The mode of action for fipronil is interference with the passage of chloride ions through the gamma-aminobutyric acid-regulated chloride channel which disrupts the arthropods central nervous system (Gant et al. 1998). Fipronil has a wide range of uses on many different pests worldwide (Yanese and Andoh 1998). This compound is considered a broad-spectrum insecticide and has been demonstrated to be effacacious against a variety of insects including termites in urban environments (Tomlin 2000).

Fipronil, as a liquid termiticide is sold asTermidor® by BASF Corporation (Research Triangle Park, NC). It is a non-repellent termiticide that can be applied as a soil barrier to protect structures from subterranean termite invasion. Subterranean termites cause damage to structures throughout the United States, and cost estimates to control and repair damage caused by these insects are as high as $11 billion annually (Su and Scheffrahn 1998, Su 2002). Compounds that are non-repellent, have a delayed reaction on subterranean termites as compared to repellent products. It has been reported that fipronil can be transferred through the colony from individual to individual by trophallaxis, grooming, and contact with contaminated soil (Kard 2003).

METHODS AND MATERIALS

Laboratory Study I

The objective of this work was to evaluate the efficacy of fipronil, in the form of Termidor® HE 0.06% AI and Termidor® HE 0.12% AI in glass tube bioassays against *R. flavipes* and *C. formosanus* subterranean termites. Arenas consisted of glass tubes measuring 15 X 1.5 cm which were capped on both ends with aluminum foil (Fig. 1) (Gold *et al*. 1994). The following treatments were utilized in this study; Termidor® HE (0.06%) and Termidor® HE(0.12%), and untreated controls (water only). Treatments to soil were made according to the manufacturer's label, and untreated controls were identically 'treated', but with water only. Equal volumes of Termidor® HE and the HE technology additive were thoroughly mixed and diluted at a rate equivalent to 5.75 mL (0.06%) solution and 11.75 mL (0.12%) solution in

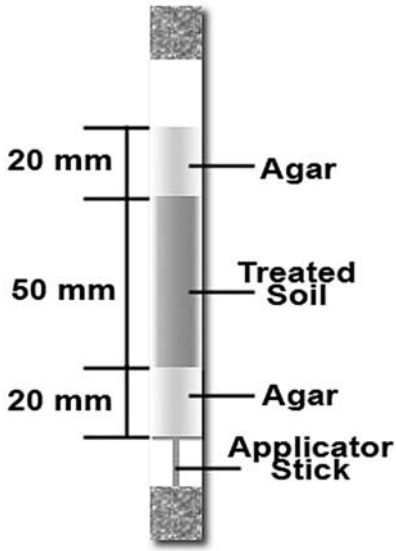

Fig. 1. Schematic showing components of glass-tube arenas used in FST feeding cessation experiment (.Waare (2000).

946 mL of water. Soil was treated with the equivalent rate of 4 L per 3 m per 0.3 m of depth. Twenty worker termites and two soldiers of either *R. flavipes* or *C. formosanus* were added to each arena after assembly. Soldier termites were added to stimulate colony dynamics and stimulate termite acticity. Post-treatment observations were made daily for 7 d. Four replications of each treatment and untreated controls were constructed for this trial. Data collected included distance tunneled and mortality, and these data were analyzed via Analysis of Variance (ANOVA) at $P < 0.05$ and means separated using Tukey's HSD (Honest Significant Difference) test at $P < 0.05$ (SPSS for Windows, V. 18.0).

Laboratory Study II

The objective of this work was to determine collateral transfer effects, if any, of Termidor® HE 0.125% AI treated soil utilizing varying ratios (donor:recipient) of *R. flavipes* and *C. formosanus* subterranean termite species through time. Recently collected termites were utilized in these trials and they were allowed to adjust to the laboratory conditions for at least 48 hrs before beginning study. Worker termites were placed in labeled Petri dishes that corresponded to a final untreated arena to allow observation of collateral effects. These worker termites of both species were marked using methods similar to Forschler 1994 with Rust-O-Leum® (Vernon Hills, IL) orange fluorescent paint. Marked termites were allowed to adjust to laboratory environment for 48 h. Treatments to the soil were made with the following products; Termidor® SC (BAS 350 95 I) Lot No. 1219502FI and Termidor® HE technology additive (BAS 270 00 S) Lot No. 502012. These products were used to create the Termidor HE Co-pack and were mixed just prior to study initiation. Treatments to soil were made according to the manufacturer's

label, and were identical to treatments in the Laboratory Study I (described above). Untreated controls were 'treated' with water only. Both the treated and the untreated control soils were allowed to dry for 24 h prior to exposing of termites to the soils. Treatment arenas consisted of Petri dishes measuring 9.0 x 2.5 cm. Approximately 34 g of treated soil was placed in each Petri dish. Worker termites of either *R. flavipes* or *C. formosanus* were added to each arena after assembly, and were allowed to contact treated soil or untreated control sand for 30 min. After the contact period elapsed, these termites (donors), were placed in an untreated, clean Petri dish (9.0 x 2.5 cm) with moistened #4 Whatman® (Maidstone, England) filter paper, and unexposed termites (recipients). Four replications of each of five donor:recipient ratios and untreated controls were constructed for this trial. The following ratios of donor:recipient termite cohorts were utilized in these trials 20:0, 15:5, 10:10, 5:15, 1:19, 0:20 (untreated marked control), and 0:20 (untreated unmarked control). Two soldier termites of corresponding species were placed in all arenas to simulate colony dynamics and stimulate worker termite activity. Observations of worker termite mortality (donors and recipients) were made at 1, 4 and 24 hours after donor:recipient mixing, then daily until 100% mortality was reached in each treatment. Data were analyzed statistically via Analysis of Variance (ANOVA) at $P < 0.05$ and means separated using Tukey's HSD (Honest Significant Difference) test at $P < 0.05$ (SPSS for Windows, V. 18.0).

Field Study

The objective of this field study was to determine effectiveness, if any, of Termidor® HE against two species of subterranean termites when applied as a post-construction treatment to infested structures. Eleven structures infested with *R. flavipes* and one infested with C. *formosanus* were utilized in this field study. All structures were located in the Houston/Galveston, TX area. Soldier termites were collected from all 12 structures, identified with termite identification keys (Scheffrahn and Hope 1996), and stored in 100% ethanol as voucher specimens. Structure owners were interviewed to verify that none of the 12 structures had received a subterranean termite treatment within the past 12 months prior to the Termidor® HE treatments. Four of the structures were pier and beam construction, which included one

structure infested with *C. formosanus*, the other eight structures consisted of monolithic slab construction. All 12 structures had at least one active subterranean termite mud tube leading from the soil into the structure that was associated with an exterior wall. A diagram was made of each structure prior to treatment to include all known points of subterranean termite infestation. All subterranean termite mud tubes were documented and marked relative to a permanent benchmark (ie. corner of the foundation).

Under the supervision of personnel from the Center for Urban and Structural Entomology and BASF, all Termidor® HE applications were made by a licensed pest management professional. All termiticide applications were made following the Termidor® SC Exterior Perimeter/Localized Interior directions for use according to the label with the following exceptions: all exterior drilling was done on 46 cm centers and all trenches were 5 X 10 cm. Volume and concentration of finished dilution applied varied according to the treatment specifications described below. At each of the structures, one half of the desired volume of water was added to the tank and then the appropriate volume of Termidor® HE was introduced to the tank, and the remaining volume of water was then added to ensure thorough mixing of the dilution. In setting up for the study, the linear length for each structure was measured prior to the treatment to ensure the proper volume of finished dilution was applied. Six structures each received one of the following treatments;

1. Termidor® HE 0.06% AI applied at 7.5 L of finished dilution/3 linear m /0.30 m of depth.

2. Termidor® HE 0.12% AI applied at 7.5 L of finished dilution/3 linear m /0.30 m of depth.

There were no interior applications made at any of the 12 structures. A Great Plains Industry Inc. 01N Series (Wichita, KS) digital flow meter was utilized during this study to measure volumes of termiticide applied at each structure. All structures were treated between August 10 and August 13, 2009. Post-treatment inspections were made on or about 1, 3, 6, 12, 24, and 36 months. Post-treatment inspections included visual assessment as well as the use of mechanical tools such as a Termatrac®, borescope, and/or infrared camera.

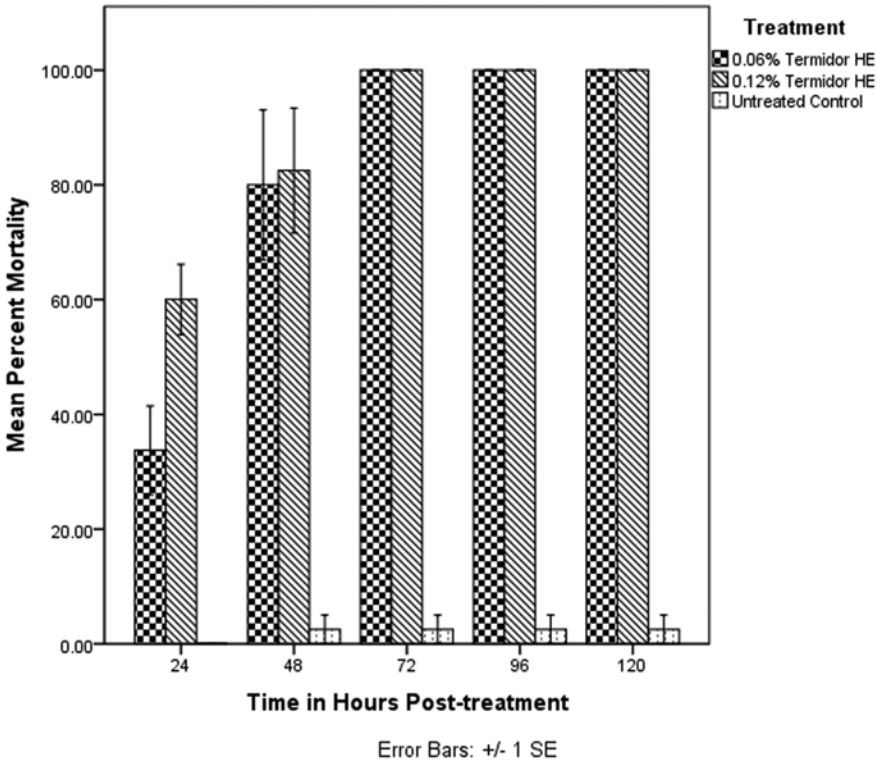

Fig. 2. Mean percent mortality of *Coptotermes formosanus* through time when exposed to soils treated with Termidor® HE.

RESULTS

Laboratory Study I

Termidor® HE 0.06% and Termidor HE® 0.12% both caused 100% mortality against both species of termites by 72 h post-treatment (Table 1). Mean mortality associated with the untreated controls was significantly less than ($P < 0.01$) from all three treatments at the end of the trials (Figs. 2 and 3), and was < 10% in both species.

In the Termidor® HE 0.06% treatment *C. formosanus* tunneled a mean distance of 10.0±7.6 mm and *R. flavipes* tunneled 7.8±2.2 mm (Table 1 and Figs. 4 and 5). This tunneling distances in the Termidor® HE 0.12% AI treatment were *C. formosanus* 16.0±3.9 mm for *C. formosanus* and 8.3±4.6 mm

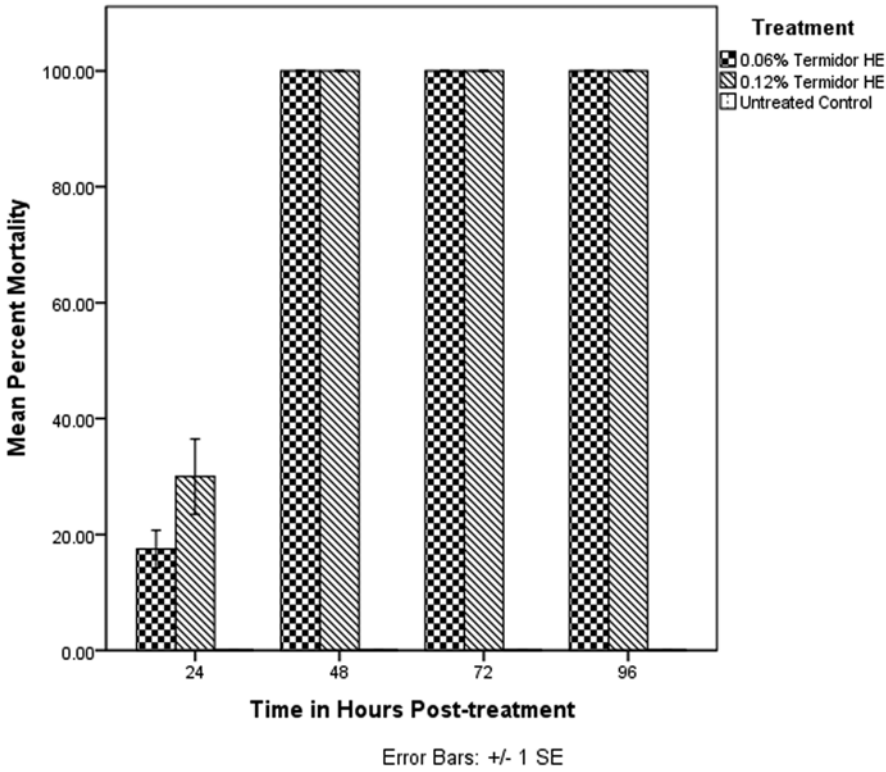

Fig. 3. Mean percent mortality of *Reticulitermes flavipes* through time when exposed to soils treated with Termidor® HE.

R. flavipes (Table 1 and Figs. 4 and 5). Both termite species in the untreated control groups tunneled the maximum distance of 50 mm, and there were significant differences ($P < 0.05$) in the distance tunneled by both species of termites in the treatments compared to the untreated controls at the end of the trial.

Laboratory Study II

Coptotermes formosanus- At the 1 h observation period for *C. formosanus*, the 10:10 donor:recipient cohort was the only one that demonstrated mortality, but it was not significantly different from the other treatments or untreated controls (Table 2 and Figs. 6 and 7). There were significant differences ($P < 0.05$) in total (donors:recipients) mortality beginning at the 4 h observa-

Table 1. Mean percent mortality and distance tunneled by *Coptotermes formosanus* and *Reticulitermes flavipes* in soils treated with Termidor® HE Co-pack (two concentrations).

Treatment	% Mortality		Distance Tunneled (mm)***	
	*C. formosanus**	*R. flavipes***	*C. formosanus*	*R. flavipes*
Termidor HE 0.06%	100.0 a	100.0 a	10.0 b	7.8 b
Termidor HE 0.12%	100.0 a	100.0 a	16.0 b	8.3 b
Untreated Control	0.0 b	5.0 b	50.0 a	50.0 a
P-values	<0.01	<0.01	<0.01	<0.01
F-stats	1521.00	1574.33	101.79	197.30
df	15	15	15	15

*All treatments caused 100% mortality in the *C. formosanus* by 120 h post-treatment.
**All treatments caused 100% mortality in the *R. flavipes* by 96 h post-treatment.
***Maximum distance tunneled could not exceed 50 mm.
Means followed by the same letter in the same column were not significantly different (P=0.05) as per Tukey's Honest Significant Difference (HSD).

tion period, the 10:10 cohort showed a significant difference (P<0.01) from the rest of the treatments and the untreated controls (Table 2 and Figs. 6 and 7). Starting at the 72 h observation, all of the effects of the treatments were significantly different (P <0.05) from the untreated controls (Table 2 and Figs. 6 and 7). At the 1 and 4 h observation periods, the only donor populations with any mortality were in the 10:10 cohort (Fig. 6). At the 72 h observation, all donor populations in the treatments had 100% mortality and the untreated controls had less than 10% mortality (Fig. 6). At the 1 h observation, there was no mortality in the recipient populations in the treatments (Fig. 7). At the 96 h observation, all recipient populations in the treatments had 100% mortality and were significantly different (P <0.05) from the untreated controls, which had less than 10% mortality throughout the study (Fig. 7).

Reticulitermes flavipes

At the 1h observation period, there were no significant differences in mortality between the treatments and the untreated controls (Table 3). At the 4 h observation period the 20:0 cohort showed the greatest mortality followed by the 15:5 and then the 10:10 cohorts, respectively (Table 3 and Figs. 8 and 9). These three cohorts were significantly different (P <0.01)

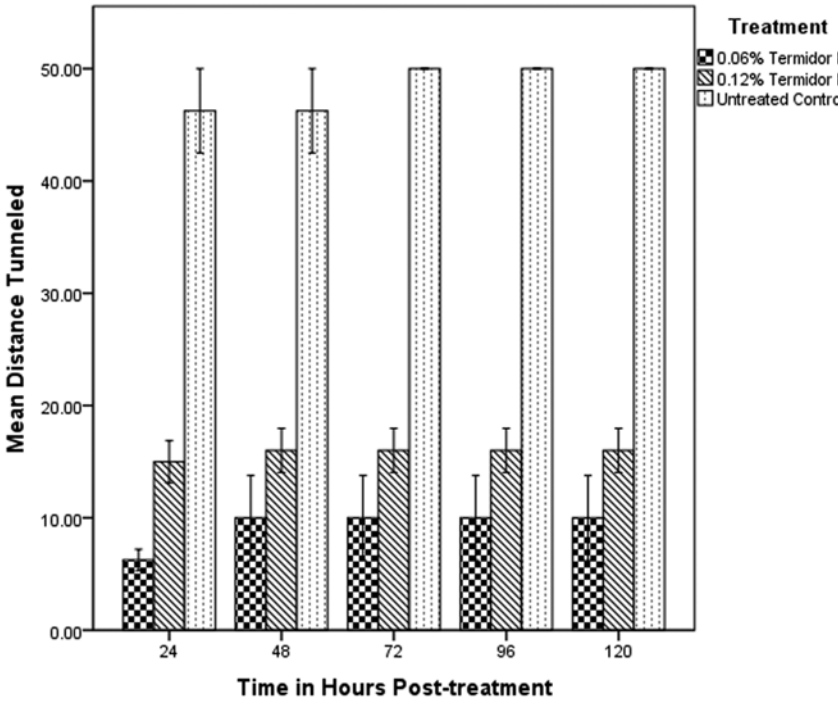

Fig. 4. Mean distance tunneled (mm) by *Coptotermes formosanus* in a glass tube bioassay through time in soils treated with Termidor® HE (maximum distance tunneled could not exceed 50 mm).

from all of the other cohorts, and the untreated controls. By the 48 h observation, all of the treatments were significantly different ($P < 0.05$) from the untreated controls (Table 3 and Figs. 8 and 9). At the 1 h observation period, there was no mortality in the donor populations in any of the treatments (Fig.8). At the 48 h observation all of the donor populations in all the treatments showed 100% mortality, and were significantly different ($P < 0.01$) from the untreated controls (Fig. 8). At the 1 h observation period there was no mortality in the recipient population in any of the treatments (Fig. 9). At the 48 h observation period the recipient populations in the 15:5 cohort was 100%, and at the 72 h observation period mean mortality of all recipient populations in the treatments was 100%. At the 72 h observation, all of the recipient populations were significantly different ($P < 0.01$) from

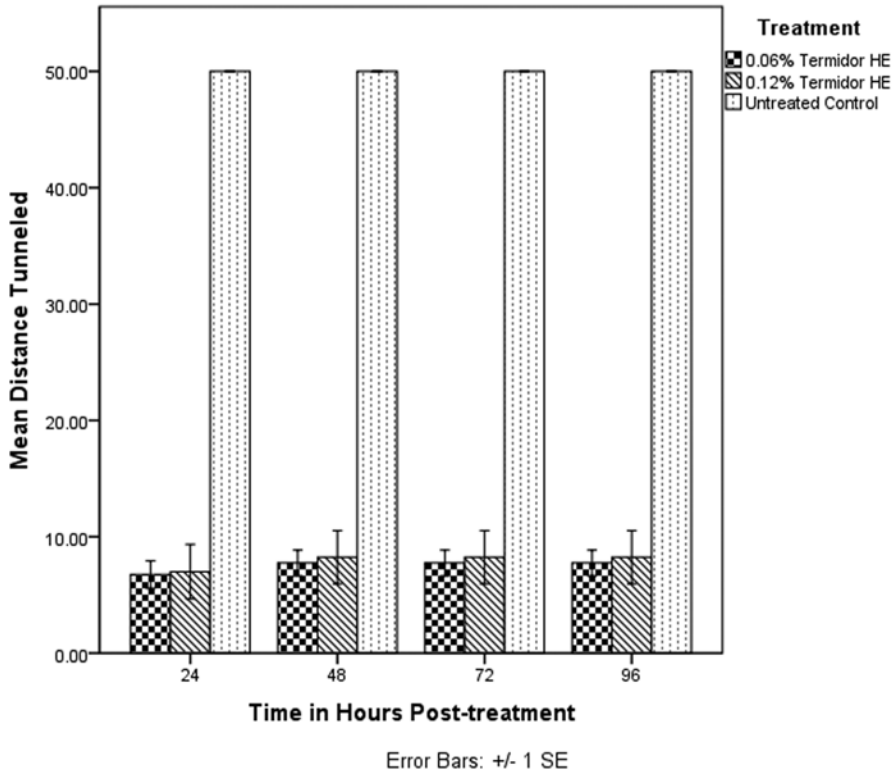

Fig. 5. Mean distance tunneled (mm) by *Reticulitermes flavipes* in a glass tube bioassay through time in soils treated with Termidor® HE (maximum distance tunneled could not exceed 50 mm).

the untreated controls, which had less than 10% mortality throughout the study (Fig. 9).

Field Study

The mean perimeter length of structures in this test was 57.8±18.6 m. The mean number of pre-trial termite mud tubes per structure was 2.3±8. The mean volume of finished solution applied to each structure was 162.7±52.3 L. There was no detection of subterranean termite activity on any of the 12 structures at any time post-treatment through 36 months.

DISCUSSION

Exposure of *R. flavipes* and *C. formosanus* to Termidor® HE treated soils

Table 2. Total mean percent mortality (both donors and recipients combined) of different cohorts of donor:recipient *Coptotermes formosanus* when donors were exposed to soil treated with Termidor® HE at 0.12% AI through time.

Cohort	Hours Post-treatment 1	4	24	48	72	96
0:20*	0.00 a	0.00 b	0.00 b	0.00 c	1.25 b	2.50 b
1:19	0.00 a	0.00 b	1.25 b	5.00 c	87.50 a	100.00 a
5:15	0.00 a	0.00 b	1.25 b	45.00 b	90.00 a	100.00 a
10:10	2.50 a	5.00 a	13.75 b	92.50 a	100.00 a	100.00 a
15:5	0.00 a	0.00 b	75.00 a	96.25 a	100.00 a	100.00
20:0	0.00 a	0.00 b	80.00 a	100.00 a	100.00 a	100.00 a
0:20**	0.00 a	0.00 b	0.00 b	2.50 c	2.50 b	5.00 b
P-values	0.28	<0.01	<0.01	<0.01	<0.01	<0.01
F-stats	3.00	6.00	53.47	201.76	246.94	1482.60
df	27	27	27	27	27	27

*untreated unmarked control
**untreated marked control
Means followed by the same letter in the same column are not significantly different P=0.05.

resulted in significantly greater mortality than untreated soils. The distance tunneled by both termite species was also statistically less in treated soils than in untreated control soils. The results show that Termidor® HE was efficacious against *R. flavipes* and *C. formosanus*.

Termidor® HE 0.06% and 0.12% caused 100% mortality to both *R. flavipes* and in *C. formosanus* at 72 hrs post-treatment, respectively. *Coptotermes formosanus* had a slower response to the treatments than *R. flavipes* at 24 and 48 hrs. The treatments caused 100% mortality, while the untreated controls caused less than 10% mortality for the duration of the study.

There was a positive correlation between increased donor numbers and recipient mortality, which provided evidence that there was transfer of active ingredient among nestmates. This was evident based on the fact that the treatments caused 100% mortality of donor and recipient termites, while the untreated controls caused less than 10% mortality ($P < 0.05$) for the duration of the study. Whether the transfer in this study was by grooming, trophallaxis, movement of contaminated sand, or simply contact, could not be determined

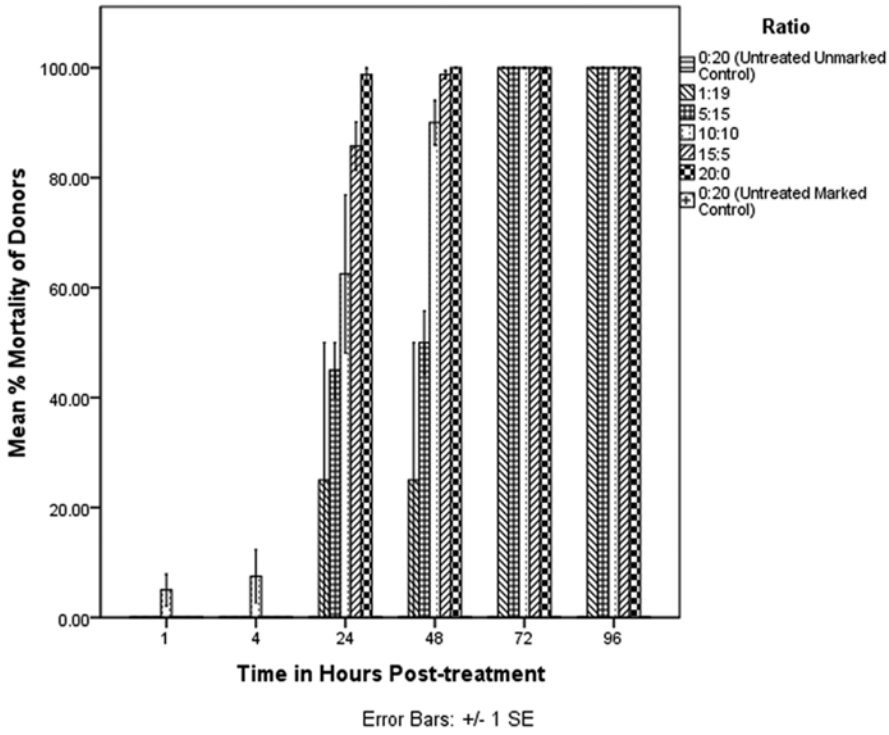

Fig. 6. Mean % mortality of donor populations in different cohorts of donor:recipient *Coptotermes formosanus* termites through time when exposed for 30 minutes to soil treated with Termidor® HE at 0.12% AI.

Table 3. Total mean percent mortality of different cohorts of donor:recipient *Reticulitermes flavipes* when donors were exposed to soil treated with Termidor® HE at 0.12% AI through time.

	Hours Post-treatment				
Cohort	1	4	24	48	72
0:20*	0.00 a	0.00 a	1.25 a	1.25 a	6.25 a
1:19	0.00 a	0.00 a	20.00 a	78.75 b	100.00 b
5:15	0.00 a	0.00 a	53.75 b	97.50 c	100.00 b
10:10	0.00 a	30.00 b	95.00 c	98.75 c	100.00 b
15:5	0.00 a	43.75 bc	100.00 c	100.00 c	100.00 b
20:0	0.00 a	57.50 c	100.00 c	100.00 c	100.00 b
0:20**	0.00 a	0.00 a	0.00 a	1.25 a	3.75 a
P-values		<0.01	<0.01	<0.01	4814.50
F-stats		19.37	42.65	150.38	<0.01
df	27	27	27	27	27

*untreated unmarked control

**untreated marked control

Means followed by the same letter in the same column are not significantly different P=0.05.

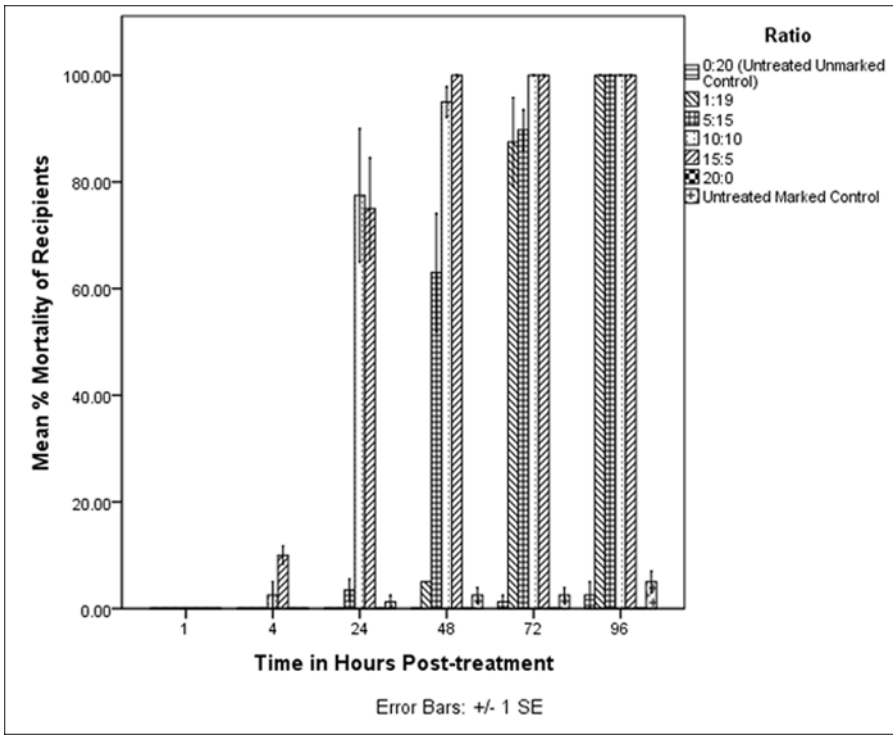

Fig. 7. Mean % mortality of recipient populations in different cohorts of donor:recipient *Coptotermes formosanus* termites through time when exposed for 30 minutes to soil treated with Termidor® HE at 0.12% AI.

and was outside of the scope of this work. All of the treatments, excluding the untreated controls, in this study caused 100% mortality in all the different ratios of donor:recipient termites in both species by 96 h post-exposure. *Coptotermes formosanus* had a slower response to the treatments than did *R. flavipes*. The higher donor:recipient ratios (20:0 and 15:5) in both species of termite were the fastest to reach 100% mortality.

In the field study, the application methods were performed by a licensed pest management professional (PMP) and were done so by the normal practices of a PMP. The application of fipronil in and around the structures was done so that applications were made on, or as close as possible to, subterranean termite entry points. This was critical when applying termiticide for control of the subterranean termites. There has been no detection of subterranean termite

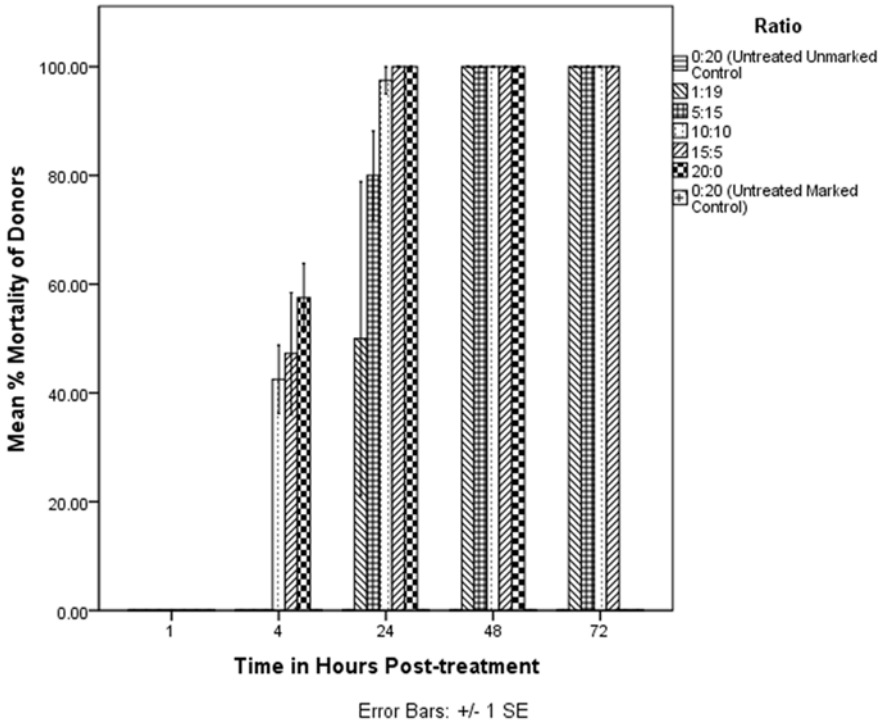

Fig. 8. Mean % mortality of donor populations in varying cohorts of donor:recipient *Reticulitermes flavipes* termites through time when donors were exposed for 30 minutes to soil treated with Termidor® HE at 0.12% AI.

activity on any of the 12 structures through 36 months post-treatment.

The results of these trials demonstrate that at either concentration, fipronil in the form of Termidor® HE is effective at causing significant mortality of *C. formosanus* and *R. flavipes* upon contact. Additionally, collateral transfer of Termidor® HE was demonstrated among both subterranean termite species investigated in these studies. Thus, Termidor® HE is not only transferred among termite nestmates, contact with the compound results in death of the insects. Given these laboratory findings, it is not surprising that when applied to termite infested structures, Termidor® HE successfully remediated all infestations and prevented re-infestation for a period of at least three years when applied at 7.5 L per 3 m per 0.3 m depth.

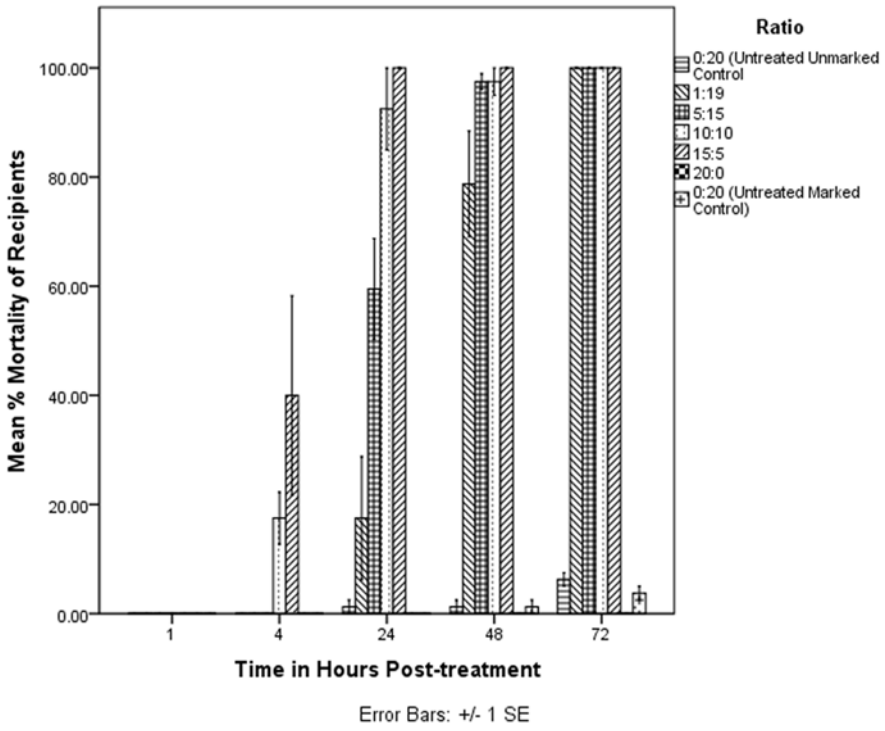

Fig. 9. Mean % mortality of recipient populations in varying cohorts of donor:recipient *Reticulitermes flavipes* termites through time when exposed for 30 minutes to soil treated with Termidor® HE at 0.12% AI.

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