Toxicity of Fipronil in Mississippi Soil Types Against *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)

by

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ABSTRACT

Three soils (a silt loam, loamy sand, sandy loam) found in Mississippi and pure silica sand were treated with fipronil and bioassayed using eastern subterranean termites, *Reticulitermes flavipes*. Soils were treated with aqueous solutions of Termidor (fipronil) at concentrations of 0, 0.12, 0.25, 2.5, 5.0 and 20.0 ppm (wt AI: wt soil) that brought the soils to 15% moisture. Estimated lethal concentrations (ppm) required to kill 50% of termite workers within 96 h after placement on the soils were: 0.49 (sandy loam), 0.70 (sand), 4.21 (loamy sand), and 6.99 (silt loam). Termite mortality decreased with increases in organic matter content of the soils treated with fipronil.

Keywords: termites, termiticide, soil type.

INTRODUCTION

Prevention of termite infestation generally requires the application of insecticides to soil under and surrounding structures. Such applications are expected to prevent termite damage to structures for a minimum of 5 years. To achieve this amount of protection against termites requires insecticides that are stable in soils that vary widely in sand, silt, clay, organic matter, and pH. Persistence of biological activity of an insecticide is dependent on the rate at which the insecticide is degraded by chemical or microbiological means. This rate of degradation differs according to soil type (Harris 1969). The effect of soil type on termiticide performance against subterranean termites has been investigated in studies in Georgia and Texas. In Georgia, Forschler & Townsend (1996) tested six termiticides, five repellents and one nonrepellent (chlorpyrifos), in four different soil types using constant exposure bioassays. Estimated lethal concentrations for all termiticides were at least 7 times

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lower in sand compared with sandy loam or sandy clay loam soils. Gold *et al.* (1996) determined persistence and toxicity of six registered termiticides, five repellents and one nonrepellent (chlorpyrifos), in five soils in Texas over 5 y. Termiticides were longer lasting and more effective in soils that were acidic with low clay and high organic content. Smith and Rust (1993) demonstrated that soils containing high concentrations of clay (10 or 20%) bind chlorpyrifos, making it less detectable and less toxic to tunneling termites. Kamble and Saran (2005) investigated the effect of concentration on the adsorption of bifenthrin, fipronil, and imidacloprid to soil. Bifenthrin and fipronil were classified as immobile in soil with organic carbon partition coefficients (K_{oc}) >2,000; while imidacloprid was categorized as having intermediate mobility (K_{oc} 150-500). All three termiticides showed decreases in adsorption coefficients (K_d) with increases in concentration applied to a loam soil low in organic matter (0.91%). Therefore, the bioavailability of these compounds to termites should increase with increasing concentration.

Several termiticides registered within the past 10 y have been classified as nonrepellent chemicals because they do not repel termites at the concentrations used, unlike pyrethroid termiticides which do repel termites. Termites entering a chemical barrier consisting of a nonrepellent termiticide receive a dose of termiticide, the efficacy of which is dependent upon bioavailability in soil.

The efficacy of fipronil in different soil types against subterranean termites has not been investigated. However, the toxicity of fipronil to subterranean termites has been documented by several researchers. The LT_{50} of fipronil to *Reticulitermes virginicus* was determined by Osbrink *et al.* (2001). Termite workers placed on filter paper treated with 630.65 $\mu g/cm^2$ of fipronil had an average LT_{50} of 271 min compared to an average LT_{50} of 13 min for workers exposed to 526.13 $\mu g/cm^2$ of chlorpyrifos. Ibrahim et al. (2003) determined the 72 h LD_{50} of fipronil to be 1.36 ng/insect in topical bioassays using *Coptotermes formosanus* Shiraki. Remmen and Su (2005) obtained an LC_{50} of 0.04 ppm after *R. flavipes* workers were exposed to fipronil treated sand for 1 wk. Hu (2005) in penetration bioassays of fipronil tested against eastern subterranean and Formosan termites found 100% mortality of both species at treatment concentrations of 50 and 100 ppm within 3 d and at 1 ppm after 28 d. Her results also showed that penetration into 50 mm thicknesses of treated sand decreased with increasing concentration. Shelton and Grace (2003) in a simple donor-recipient test exposed *C. formosanus* workers to sand treated with fipronil at 1, 10, and 100 ppm for 1 h. Mean mortalities of donors after 14 d were 36, 36, and 98%, respectively.

The objective of this study was to determine the effect of three soil types found in Mississippi on the toxicity of fipronil to subterranean termites in laboratory bioassays.

MATERIALS AND METHODS

Soils used were from three counties in Mississippi: Harrison, Oktibbeha, and Winston. Soils were collected in 19-L buckets from Harrison and Oktibbeha Counties and brought to the laboratory. The soil from Winston County was obtained locally from a commercial source and is commonly used as fill under building foundations. Samples of these soils were analyzed by the Soil Testing Laboratory at Mississippi State University. Each soil type was treated 20 – 24 h before bioassays with aqueous solutions of fipronil (Termidor 80 WG) at 0, 0.25, 0.5, 1.0, and 2.5 ppm (wt AI : wt soil). Pure silica sand, 40-100 mesh (Fisher), served as a standard for the test. Termiticide solutions were prepared to obtain the required concentration in a volume of deionized water necessary to bring 200 g of oven-dried soil to 15% (wt : wt) soil moisture. Thirty ml of termiticide solution was poured into clear plastic bags containing 200 g of each soil type and mixed thoroughly. Treated soil from each bag was placed in four plastic petri dishes (60 × 15 mm) that were filled to approximately ⅔ capacity. These four dishes constituted a replicate. Water-only control soils received deionized water only.

Termites, *R. flavipes*, were collected from fallen pine logs on the Noxubee National Wildlife Refuge, Brooksville, MS, and held at ambient temperature in galvanized trash cans in the laboratory. All colonies used had been collected within 3 mo of the study. Twenty-five termite workers were placed in each petri dish containing moist, treated soil. Small pieces of filter paper (Whatman 1, ca. 3.8 cm^2) were placed in petri dishes 1 h after introduction of termites to provide food for termites during bioassays. Petri dishes were held at 25°C during bioassays.

The degree of toxicity (ataxia, morbidity, and death) was recorded after 72 h. Termites that were ataxic ranged from being unsteady on their feet to lying on their backs wildly kicking their legs. Termites on their backs that were barely moving or showed any movement, however slight, when prodded were recorded as moribund; those that did not move when prodded were dead.

Experimental design was a randomized complete block with 3 replicates per concentration and soil type. A different termite colony was used for each replicate. The toxicity (LC_{50}) of fipronil was determined for each soil type using PROC GLMMIX (Littell *et al.* 2006) by regressing the logit of mortality on log concentration + 0.5. Fixed effects in the model were soil type, concentration, and soil type*concentration. Colony, dish within colony, soil type, and concentration were random effects. Mortality data were subjected to an analysis of variance using PROC GLMMIX. Fixed and random effects were as previously indicated but with concentration taken as a classification variable. Least-square means were separated using the LINES option.

RESULTS AND DISCUSSION

The results of the soil analysis are given in Table 1. The silt loam, loamy sand, and sandy loam had similar pH's which ranged from 4.4 – 4.8, while that of the sand was slightly higher (6.5). Percentage organic matter was highest in the silt loam (2.65%), intermediate in the loamy sand (1.82%), and low in the sandy loam (0.33%), and lowest in the sand (0.13%). Percentage clay was highest in the silt loam (15.00%), followed by the sand loam (7.50%), loamy sand (2.20%), and sand (0.00%). Silt content was highest in the silt loam (72.25%) followed by the loamy sand (20.00%), the sandy loam (18.75%), and sand (5.25%). Percentage sand was highest in the sand (94.72%), similar between the loamy sand (78.00%) and the sandy loam (73.75%) and lowest in the silt loam (12.75%).

When the logit of percentage mortality on log concentration (+0.5) was calculated lethal concentrations of fipronil that kill 50% of termites

Table 1. Analysis of Mississippi soil types used in 96 h constant exposure bioassays.

Soil	pH	%OM	%Clay	%Silt	%Sand
Silt Loam	4.8	2.65	15.00	72.25	12.75
Loamy Sand	4.4	1.82	2.20	20.00	78.00
Sandy Loam	4.7	0.33	7.50	18.75	73.75
Sand	6.5	0.13	0.00	5.25	94.75

(LC_{50}) were 0.49 and 0.70 for the sandy loam and sand, respectively (Table 2). The LC_{50} values of the loamy sand and the silt loam were 4.21 and 6.99, respectively. Fipronil was approximately 7 and 12 times less toxic in loamy sand and silt loam, respectively, than in sand and sandy loam. Remmen & Su (2005) exposed *C. formosanus* workers to fipronil treated sand for 1 wk and obtained an LC_{50} of 0.04 ppm which is 17.5 times lower than the LC_{50} that was obtained in this study but Remmen & Su's bioassay was 3 d longer.

The ranking of the LC_{50} values obtained in this study corresponds most closely to the amount of organic matter (OM) in the soil. Fipronil efficacy, and thus bioavailability, decreased as the percentage of organic matter in the soil increased. This result is corroborated by results of Bobe et al. (1997) who showed that fipronil adsorption was correlated with the OM content of soil. The effect of soil type on termiticide toxicity was also investigated by Forschler and Townsend (1996) in Georgia. Determination of LC_{50}'s of termiticides in 48 h bioassays by showed that LC_{50} values for different termiticides in a sandy loam with 3.75% OM ranged from 6 to 102 times greater than in sand that contained <0.07% OM. In our study, the LC_{50} value for fipronil in loamy sand that contained 1.82% OM was 6 times higher than that in sand with 0.13% OM.

Results of the analysis of variance for percentage mortality showed highly significant main effects for soil ($F_{3,264} = 57.65$; $P < 0.0001$) and concentration ($F_{5,264} = 86.82$; $P<0.0001$). Mean percentage mortalities for the different soils averaged over concentrations followed the order: 25.0 ± 4.0 (silt loam) < 33.1 ± 4.6 (loamy sand) < 62.7 ± 5.1 (sand) = 64.3 ± 4.6 (sandy loam). Mean percentage mortalities for the different concentrations averaged over soils followed the order: 3.9 ± 2.1 (0 ppm) < 20.9 ± 4.3 (0.12 ppm) = 28.5 ±

Table 2. LC_{50} values (95% C.L.) and slopes (± SE) from logistic regressions of percentage mortality on log concentration (+ 0.5) from bioassays of fipronil treated soils.

Soil	N	Slope ± SE	LC_{50} (95% C.L.)	*P*
Silt Loam	300	2.2881 ± 0.2401	6.99 (3.00 – 15.54)	0.0001
Loamy Sand	300	2.7003 ± 0.2516	4.21 (2.04 – 8.23)	0.0001
Sandy Loam	300	1.8386 ± 0.2289	0.49 (0.0 – 1.91)[a]	0.0001
Sand	300	2.2995 ± 0.321	0.70 (0.16 – 1.66)	0.0001

[a]Lower confidence limit was negative and thus was set to zero.

5.6 (0.25 ppm) < 61.2 ± 5.6 (2.5 ppm) < 72.3 ± 4.5 (5.0 ppm) < 90.9 ± 2.0 (20.0 ppm). The interaction of soil*concentration was also highly significant ($F_{15,264} = 3.74$; $P < 0.0001$). Percentage mortalities of termites on water-only treated soils (controls) were not significantly different among soil types (Table 3). Percentage mortality on the silt loam and loamy sand was different from the control only when the concentration of fipronil was increased to 2.5 ppm. The sandy loam and sand had significantly higher mortalities than the silt loam and loamy sand at all fipronil concentrations, except at 20.0 ppm. Termite mortality on sand treated with 20.0 ppm was significantly greater than the other soils treated at the same concentration. Termite mortalities on treated sand and sandy loam were significantly higher than controls at all concentration. Sand and the sandy loam had equivalent mortalities at all except 20.0 ppm where mortality was significantly higher on sand than on sandy loam. However, mortality on sand treated with 20.0 ppm was not higher than that on sandy loam treated with 5.0 ppm.

This study demonstrates that fipronil had greater bioavailability in soils low in organic matter. This was most dramatically shown in the sandy loam treated with fipronil in this study. Fipronil in this soil, which had an organic matter percentage of 0.33%, had the lowest LC_{50} of the three soils tested. Interestingly, this soil is commonly used as fill beneath building foundations. Providing fill using this soil type not only insures a stable foundation for structures, it also enhances the efficacy of fipronil against termites. The results of this study also highlight the importance of minimizing the amount of organic matter

Table 3. Mean percentage mortality (± SEM) of termites in bioassays (96 h) of different soil types treated with fiponil.

	Soil			
Concentration (ppm)	Silt Loam	Loamy Sand	Sandy Loam	Sand
0	2.3 ± 0.9g	0.7 ± 0.4g	3.0 ± 1.3g	9.7 ± 8.2g
0.12	1.3 ± 0.6g	1.7 ± 0.8g	45.4 ± 7.1cde	35.1 ± 11.0ef
0.25	4.1 ± 2.7g	2.7 ± 2.4g	60.7 ± 11.2cd	46.6 ± 12.8cde
2.5	18.0 ± 5.8f	43.9 ± 9.0cd	90.7 ± 5.0b	92.3 ± 2.7b
5	40.4 ± 8.9de	62.0 ± 8.4c	93.5 ± 3.0ab	93.2 ± 1.8b
20	83.8 ± 5.2b	87.8 ± 4.2b	92.5 ± 3.6b	99.3 ± 0.7a

Means followed by the same letter are not significantly different (*P*>0.05) as determined by the LINES option of PROC GLMMIX (Littell et al. 2006).

occurring around foundations where perimeter applications are made. Placing bark mulch in flower beds next to buildings not only provides food and pathways for termites but also increases the amount of organic matter in soil as the mulch decomposes, thus diminishing the bioavailability of termiticides applied along building perimeters.

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