Chapter 8

Depth of initial penetration of two aqueous termiticide formulations as a function of soil type and soil moisture

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The initial penetration of two termiticide formulations, Premise 75 (imidacloprid) and Termidor SC (fipronil), were tested in four soils at three moisture levels (5, 10 and 15% by weight) in a laboratory study. Within each soil type and moisture combination, the highest concentrations of active ingredient were found in the top 1 cm of soil and decreased with increasing depth. As soil moisture increased, active ingredient concentration in the top 1 cm decreased while active ingredient concentration in lower depths, especially 2 to 5 cm, increased. For each compound, the effect of soil type on active ingredient penetration depended on the soil moisture and soil depth, with few effects at low moisture and greater depth. Soil type had little overall effect on the penetration of either compound, however, as both compounds were contained in the top 5 cm in each situation. Both compounds were the most toxic to termites in soils with low organic matter.

Chemical soil treatment for the prevention of termite infestation in structures has been practiced since at least the late 1920s (*1*), with previous recommendations relying solely on good building practices (such as minimizing soil-wood contact) and impregnated timber (*2*). The first tests of soil chemical application were initiated in 1928 in California using termite-infested utility poles (*3*). It is interesting that chemical soil treatment, now a multi-billion dollar industry in the United States, was originally thought of as being useful only on a temporary basis (*4*) and should not substitute for good building practices. Good building practices are still recommended in addition to chemical application (*5*) and are incorporated into most building codes.

Prior to the end of the Second World War, most houses were of the wall and pier, or "conventional" foundation type. Soil treatment in this type of construction consists of trench applications, where soil is removed around a foundation wall or support piers in a trench about six inches (15 cm) wide, and then the soil is treated as it is being replaced. This method is still used for what are now referred to as "perimeter" treatments.

Following the Second World War, houses constructed on a concrete slab in direct contact with the ground began to gain in popularity (*6*) and continue to do so. According to the United States Census Bureau, 72% of all houses built in the southern United States in 2006 had slab foundations, compared with 46% in 1971, the first year for which records of this type were available. Some thought slab construction was an end to termite problems, because termites would not be able to penetrate several inches of concrete. It was believed that a perimeter treatment around the slab would prevent attacks from the edge. However, it was soon found that termites could, and did, enter structures from below through plumbing and electrical service penetrations, expansion joints and cracks (*7*). It was therefore recommended that an overall termiticide application to the soil before the slab is poured would prevent termite access through these areas.

The United States Department of Agriculture - Forest Service (USFS) was among the first to test the efficacy of this application method. Based on tests initiated in 1946, an application rate of 1 pint of insecticide formulation per square foot (4.75 liter per square meter, or 1.25 gallons per 10 square feet, more than the current label rate) was proposed in 1954 (*7*). This was adjusted to 1 gallon per 10 square feet (4 liter per 1 square meter) in 1956, for the reason than it was simpler for the applicator to calibrate spraying equipment in gallons-per-minute and use simple math to determine how much solution was needed (or for how long to run the sprayer) once the square footage was known (*8*). For example, treating 1000 square feet would require 100 gallons and take 20 minutes at five gallons per minute. The Federal Housing Administration adopted this rate as a guideline in 1958 (*9*), and it is now considered the standard industry practice.

The integrity of the chemical barrier is important to the prevention of termite infestations. In slab-type construction, shortly after a termiticide is applied, a vapor barrier is placed over the soil, reinforcing bars or mesh is laid, and concrete is poured over the vapor barrier. These processes may take place over the course of several hours to more than one day, and all of these activities raise the potential of disturbance to the chemical barrier. If the soil disturbance is great, the integrity of the chemical barrier may be compromised. A "perfect" termiticide formulation should penetrate deeply enough to provide a barrier resistant to minor disturbance but not penetrate so deeply that the compound is diluted by soil to below the level of effectiveness.

The initial soil penetration of termiticide solutions has not been examined since around 1970, when USFS personnel studied the depth of initial penetration of organochlorine termiticides (*10 – 13*). These studies determined that most of the applied insecticide remained in the top 0.75 inch (2 cm) of the soil. The active ingredients used, chlordane, aldrin, dieldrin and heptachlor, are nearly insoluble in water and practically immobile in the environment, especially under the conditions found in termite control (i.e. beneath a concrete slab) where they are protected from the elements (*14*). In some cases, organochlorine insecticides were diluted in fuel oil or kerosene (for an example, see *15*), a practice no longer used. Even when diluted in water, the concentrated forms of these products contained petroleum distillates or hydrocarbons (for examples, see *16* and *17*).

Most termiticidal active ingredients introduced since about 1970 have been more water-soluble than earlier compounds, for example permethrin (<1 mg/L), chlorpyrifos (2 mg/L), fipronil (2 mg/L) and imidacloprid (510 mg/L) (*18*). Water-soluble compounds have a greater potential than insoluble compounds to move through the soil with the application solution. This may aid in the spread of the active ingredient, resulting in a more uniform distribution in the soil due to lateral and vertical movement. Hydrophobic compounds diluted in a petroleum carrier should penetrate the soil differently than more hydrophilic compounds diluted in water. Systematic evaluations of soil penetration by aqueous solutions of newer active ingredients have not been made.

This study examines the initial depth of penetration of two aqueous termiticide formulations, Premise and Termidor in four different soils and at three soil moisture levels.

Materials and Methods

Soils

Four soil types, designated U, D, H and P were collected, reflecting different contents of clay, silt, sand, organic matter, pH, cation exchange capacity (CEC) and field capacity (Table 1). U soil was loamy sand collected from the USFS Termiticide Testing Program site in Union County, SC. D soil was silt loam collected in the John Starr Memorial Forest near Dorman Lake in Oktibbeha County, MS. H soil, a sandy loam, was collected from the USFS Termiticide Testing Program site in the Harrison Experimental Forest in Harrison County, MS. P soil was sandy loam collected from Parker Sand and Gravel Co., Lowndes County, MS and is of a type approved by local building authorities for use as construction fill. All soils were air-dried, clumps were broken apart with a hammer and each soil was sieved to remove stones and roots. The soil texture analysis, pH, organic matter and cation exchange capacity was determined by the Mississippi State University Extension Service. To approximate the water holding capacity, 50-g portions of each soil (oven-dried at 100 ºC overnight) were placed in Buchner funnels fitted with filter paper to prevent loss of soil. Distilled water, enough to thoroughly wet each soil, was added and a 34.5 kPa (5 psi) vacuum was applied until water was no longer observed dripping from the funnel. The soils were re-weighed and the water content was calculated (*19*).

Table 1. Properties of soils used in this study

Soil Type	Texture	Silt (%)	Sand (%)	Clay (%)	pH	%OM[a]	CEC[b]	Field Capacity (%)
U	Loamy Sand	19.75	77.75	2.50	5.2	1.41	4.10	16.6
D	Silt Loam	50.00	42.50	7.50	5.3	2.43	15.20	35.9
P	Sandy Loam	40.00	55.00	5.00	5.1	0.52	6.00	21.2
H	Sandy Loam	27.75	69.75	2.50	5.0	2.17	4.50	17.6

[a] Percentage organic matter
[b] Cation exchange capacity

To hydrate each soil for the test, the mass (± 0.1 kg) of each soil in a 19 liter (5 gal) bucket was found, and the amount of water required to constitute three moisture levels (5, 10 and 15% by weight) was calculated. Water was added to each 19 liter soil portion in a cement mixer by using a carbon dioxide sprayer during tumbling in a cement mixer for > 5 min. The soil for each 19-liter portion was then added to six plastic buckets (18 × 14 ID) to a depth of 15 cm.

Soil treatment, extraction and analysis

Two commonly used termiticides, Termidor and Premise, were mixed at the labeled rate for sub-slab treatment (0.06% and 0.05%, respectively). The application of the termiticide solutions was conducted within two hours of soil hydration. The termiticide solution (62 mL) was applied to the soil within the plastic buckets to approximate the 4 liter/1 square meter (1 gal/10 ft^2). The solutions were applied by using a compressed air paint sprayer. Lids were placed on each bucket to prevent evaporation. After 24 hours, a 7.6 ID × 15-cm plastic pipe was pushed into the center of the treated soil, which minimized edge effects caused by the plastic buckets. The pipe was capped, then the bucket was upturned and the soil was allowed to fall out of the bucket but remain in the pipe. A 7.6-cm diameter plastic dowel was used to push the soil out of the pipe at 1-cm increments to a depth of 12 cm. Each soil increment was placed in labeled re-sealable plastic bags. The active ingredients were extracted from the soil and analyzed by procedures described below.

Imidacloprid was extracted and analyzed by a method modified from Peterson (*20*). Recovered soil (15 ± 1 g) was placed in a foil weigh boat and air dried at room temperature overnight. Dried soil (10 ± 0.5 g) was placed in a glass jar and 20 mL of 80: 20 acetonitrile: water solution was added and then the soil was then shaken for 4 hours at 200 rpm. The jars settled for > 48 hours, the liquid was decanted and vacuum filtered through glass fiber filters. The collected filtrate was analyzed for imidacloprid content on a Waters Alliance 2695 liquid chromatograph, consisting of 20 µL injection, water + acetonitrile

(60 + 40 by volume) mobile phase at 1 mL min^{-1} through a Whatman Partisphere RTF C-18 column (4.6 × 250 mm) fitted with an Agilent XDB C-18 (4.6 × 12.5 mm) guard column and UV detection (270 nm) on a Waters 996 photodiode array detector. Percentage recoveries for this method were 89, 95, 99 and 115% at 100 μg/g soil and 89, 91, 102 and 112% at 10 μg/g soil for H, U, P and D soils, respectively.

Fipronil was extracted by placing 35 ± 1 g recovered soil into a foil weigh boat and oven drying at 90 °C overnight. After cooling, 25 ± 0.01 g dried soil was extracted by using a Dionex ASE 200 accelerated solvent extractor. In this method, 60 mL of 70: 30 acetonitrile: acetone mixture is passed through the 25-g sample at 100 °C and 10342 kPa (1500 psi). The sample was concentrated to 10 mL under a nitrogen stream, and the resulting extract was analyzed by an Agilent 6890 gas chromatograph. Each injection was 1 μL. The injector temperature was 250 °C with an Agilent 1909 1A-112 ultra 1 methyl siloxane 25 m × 320 μm inside diameter × 0.52 μm film thickness column, with helium carrier gas at 20 mL/min. The oven temperature program was 50 °C for 1 min, ramped at 30 °C per minute to 200 °C and held for 10 minutes, ramped again by 30 °C per minute to 230 °C and held for 8 minutes, for a total run time of 25 min. An electron capture detector was used at 250 °C. There was a three-minute equilibration time between runs with two needles washes of hexane followed by two needle washes of acetone. Percentage recovery of fipronil by using this method was 113% for H soil, 98.3% for U soil, and 98.2% for D soil at 20 μg/g soil.

A split-plot arrangement was used in a randomized complete block design (blocked by soil type, with all treatments for a particular soil conducted on the same day), with each container (combination of soil moisture and compound) as the whole plot factor and soil depth as the subplot factor. The study had three replications. Mixed analysis of variance on SAS (*21*) was used to determine significance due to soil type, soil moisture and depth.

Termite bioassays

Stock solutions of Premise and Termidor were prepared by serial dilution. For the range finding assay, solutions were prepared so that the compounds were tested at 100, 50, 10, 1 and 0.1 μg/g soil. Each soil was separately treated by adding 10 mL of the appropriate dilution to 100 ± 0.1 g oven-dried soil in plastic bags. The soil was mixed thoroughly and allowed to sit overnight. Three 15-g portions were removed and placed in separate 15 × 60-mm ID Petri dishes. A square of cardboard, 1 × 1 cm, was placed in the dish and ten *Reticulitermes flavipes* workers were added. Survival of termites was counted in each dish at 7 days. Following the range finding assays, fipronil solutions were made to constitute 1, 0.8, 0.6, 0.4, 0.2 and 0.1 μg/g soil and imidacloprid solutions were made to constitute 10, 8, 6, 4, 2 and 1 μg/g soil. The solutions were applied as described above and termites from the same colony used for the range finding test were used in the manner described above. The LC_{50} values and 95% fiducial limits were calculated by using Probit analysis on SAS (*21*).

Results and Discussion

Depth of penetration

Data were not collected for either compound at 15% soil moisture in U soil. This soil saturates at about 16% moisture (Table 1) and standing fluid was observed on the soil surface 24 hours after application.

The effects of soil moisture on concentration were examined for each combination of soil type and compound (Figures 1 and 2). For all soil types, the effect of soil moisture on fipronil concentration depended upon depth; i.e. there was a statistically significant interaction between soil moisture and depth ($P < 0.0001$ for each soil at 14, 42 degrees of freedom for D, H and P and 7, 28 degrees of freedom for U). Fipronil concentration in the top 1 cm declined with increasing soil moisture, while fipronil concentrations at 2 to 5 cm were higher in soil of 10% moisture (Figure 1). Except for D soil at 15% soil moisture, there were no differences in fipronil concentration below 5 cm for any soil type or soil moisture level.

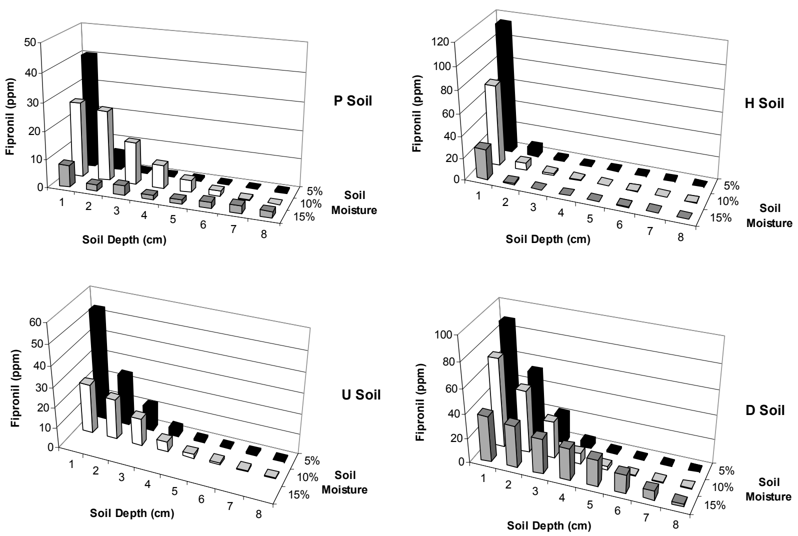

Figure 1. Fipronil recovered from each soil at each depth and moisture combination.

A pattern similar to that observed for fipronil was observed for imidacloprid (Figure 2). There was a significant interaction between soil moisture and depth for U, H and P soils ($P < 0.0001$ at 14, 42 degrees of freedom for H and P, 7 and 28 degrees of freedom for U), but depth was the only significant factor for D soil ($P < 0.0001$ at 7, 42 degrees of freedom). Similar to fipronil, the concentration of imidacloprid in the top 1 cm declined with an increase in soil moisture, although an increase was observed in P soil at 10% soil moisture. Imidacloprid concentration at 2 to 5 cm was higher for 10% soil moisture in P soil, and it was roughly equivalent among the three soil moistures in the other three soils.

Soil types were compared within compounds. For both imidacloprid and fipronil, there was a significant three-way interaction between soil type, soil moisture and depth (imidacloprid: df = 35, 147; $F = 5.56$, $P < 0.0001$; fipronil: df = 35, 154; $F = 7.22$, $P < 0.0001$). Fipronil concentrations were much lower in P and U soils than in H and D soils and the effects due to soil moisture and depth are discussed above. Imidacloprid concentrations were roughly equivalent

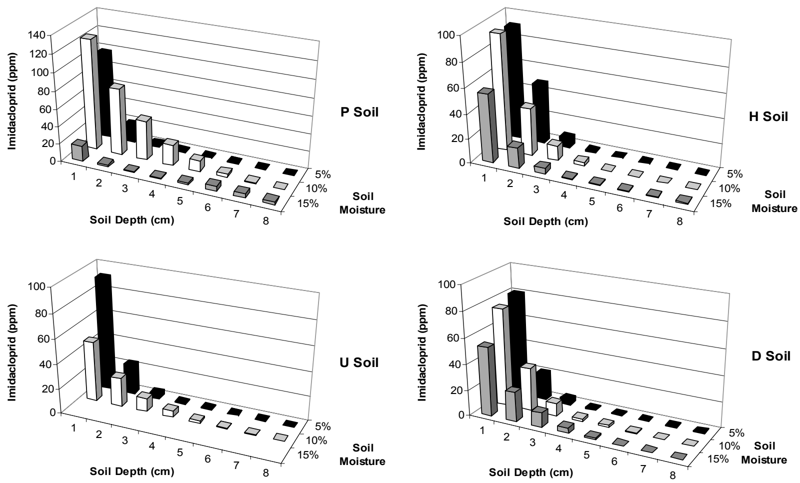

Figure 2. Imidacloprid recovered from each soil at each depth and moisture combination.

between the four soils, but with differences between P and U soils at 10% soil moisture.

Statistical interaction aside, the difference in the penetration of either compound in the soil types is not great. Neither compound penetrated much beyond 5 cm regardless of soil type or moisture, with the exception of fipronil in D soil at 15% soil moisture. Fipronil penetrated the least well into H soil, which is surprising because H soil is relatively sandy. Imidacloprid penetrated similarly into all four soils. Soil type, then, should not be a major factor affecting the initial penetration of a termiticide. Realistically, unless the local building codes require that fill dirt be brought in, soil type is not a choice and even then the fill will more likely be chosen due to expansion and settling potential than for properties conducive to termite control.

Termite bioassays

The LC_{50} values for imidacloprid and fipronil in each of the four soils are shown in Table 2. Both compounds were the most toxic in P soil, which is used as a construction fill and is therefore the most relevant for termite control beneath structures. Fipronil was of equivalent toxicity in U, H and P soils, but less toxic in D soil. D soil had the highest organic matter content of the four soils used, as well as the highest silt, clay and cation exchange capacity. Imidacloprid was equally toxic in all soil types except P soil, where it was more toxic.

Table 2. Seven-day LC_{50} values (95% FL) of fipronil and imidacloprid in μg/g soil applied to the soils used in this study to *R. flavipes*

Soil Type	Fipronil LC_{50} (95% FL)	Imidacloprid LC_{50} (95% FL)
P	0.14 (0.11, 0.17)	3.25 (1.85, 5.18)
D	0.62 (0.48, 0.75)	9.29 (6.39, 18.34)
H	0.18 (0.14, 0.23)	15.11 (12.82, 17.44)
U	0.18 (0.15, 0.21)	12.39 (10.68, 14.14)

Both compounds were the most toxic in P soil, which had the lowest organic matter. A recent study by Mulrooney and Gerard (*22*) found that among four soil types, fipronil was most toxic in a sandy loam soil, followed by sand, a loamy sand and a silt loam, and this trend generally followed a pattern of increasing organic matter. The same general pattern was observed here. Therefore, termiticide-treated soils lower in organic matter should provide the most toxic barrier to termites.

The depth of penetration determines the thickness of the chemical barrier. From these results, it seems that 10% soil moisture for P soil and 15% soil moisture for D soil would provide the thickest barrier. It is noteworthy, however, that there is a reduction in concentration in the top 1 cm with an increase in soil moisture.

This begs the question of what type of barrier is desirable? A thick barrier will withstand minor disturbances more than a thin barrier, but a thick barrier, with lower initial concentration, may degrade to below effective levels more quickly than a thin barrier with higher initial concentration. Figure 3 illustrates this with a hypothetical compound with a half-life of 6 years and that is not effective below 20 μg/g soil. If the barrier is thin, say 1 cm, and the initial concentration of this compound were 100 μg/g soil in the soil, it would take about 14 years to degrade to below 20 μg/g soil. If the initial barrier is thicker, say 3 cm instead of 1 cm, the initial concentration would be lower, here starting at about 60 μg/g soil. In this situation, the barrier would degrade to below 20 μg/g soil in 10 years instead of the 14 years required for the thinner barrier.

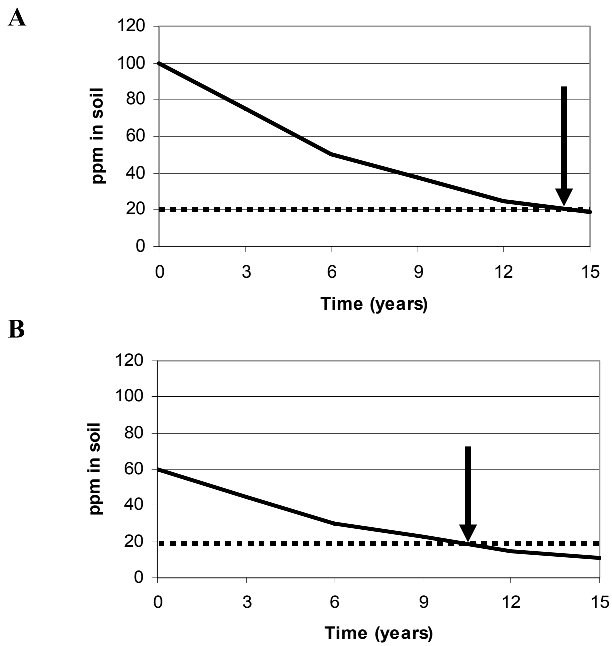

Figure 3. Degradation of a hypothetical compound with a half-life of 6 years and minimum effective concentration of 20 μg/g soil (dashed line). Arrow indicates time when treatment is no longer effective for A) a 1-cm barrier of high initial concentration and B) a 3-cm barrier of lower initial concentration.

Further studies are currently underway to determine how application volume affects the initial thickness of the barrier. Longer-term field studies are necessary to determine how chemical barriers of different thickness affect structural protection.

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