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Authors: Sapkota, Rajani, Stout, Michael J., and Henderson, Gregg

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# Residual Effects of Termiticides on Mortality of Formosan Subterranean Termite (Isoptera: Rhinotermitidae) on Substrates Subjected to Flooding

**Rajani Sapkota,[1,2,3] Michael J. Stout,[1] and Gregg Henderson[1]**

[1]Department of Entomology, Louisiana State University Agricultural Center, Baton Rouge, LA 70808, [2]Current address: Department of Entomology, Purdue University, 901 W State Street, West Lafayette, IN 47907, and [3]Corresponding author: rajani.sapkota4801@gmail.com

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## Abstract

Concerns on efficacies of termiticides used for soil treatment to prevent Formosan subterranean termite (*Coptotermes formosanus* Shiraki) infestations have prompted pest control companies to suggest that retreatments are necessary after flooding of homes. Therefore, to address concerns about the efficacy of termiticides after flooding, we designed a flooding simulation experiment in the laboratory. We used four formulated termiticides containing fipronil, imidacloprid, chlorantraniliprole, or bifenthrin as active ingredients (a.i.) and two colonies of field-collected *C. formosanus* for this study. Evaluations of each chemical at concentrations of 1, 10, and 25 ppm in both sand and soil were conducted in the laboratory by comparing termite mortalities in no-choice bioassays after exposure to flooded (for 1 wk) and unflooded substrates. Toxicity from bifenthrin and fipronil were not affected by flooding regardless of substrate type except at the lowest concentration tested. Toxicity from chlorantraniliprole was lower in flooded sand at 1 ppm but otherwise similar among flooding treatments. In flooded soil, toxicity from chlorantraniliprole was low at 1 ppm, but unexpectedly high in flooded conditions at 10 and 25 ppm. For all concentrations of imidacloprid-treated sand, mortality of *C. formosanus* was reduced after a flood. However, like chlorantraniliprole, 10 and 25 ppm of imidacloprid-treated soil in flooded conditions resulted in an increased toxicity on *C. formosanus*. Our study supports the idea that chemicals with a higher water solubility like imidacloprid may require a home to be retreated with less water-soluble termiticides or baits after a flood.

**Key words:** termiticide efficacy and flooding, fipronil, imidacloprid, bifenthrin, chlorantraniliprole

The Formosan subterranean termite, *Coptotermes formosanus* Shiraki, is a ground-dwelling termite that aggressively attacks cellulose food resources (Spink 1967). Annual costs incurred by consumers for preventive and remedial management of *C. formosanus* exceed US$1 billion (Lax and Osbrink 2003). Considerable damage can be observed in a short period after initial infestation because of a large colony size, which may range between 2 and 10 million individuals (Yates and Tamashiro 1999). Control measures that include physical, biological, and chemical tactics have been developed against *C. formosanus*. A variety of insecticidal active ingredients are used for soil treatment and baiting in current termite control programs (Su 2002, Gautam and Henderson 2014).

Treatment of soil with liquid termiticides is one of the widely used methods for the management of subterranean termites (Smith and Rust 1990, Racke et al. 1994, Su and Scheffrahn 1998, Peterson et al. 2006). Liquid termiticides provide a chemical 'barrier' against termites (Horwood et al. 2010). Some of the active ingredients used in liquid formulations include fipronil, bifenthrin, chlorantraniliprole, cyantraniliprole, imidacloprid, chlorfenapyr, and indoxacarb (Mao et al. 2011). Fipronil is considered a nonrepellent termiticide because it does not cause immediate repellence from a treated area (Hu 2005, Yeoh et al. 2006, Yeoh and Lee 2007), although it may be repellent at its highest label rate of 0.125% in treated sand (Ibrahim et al. 2003). Nonrepellent termiticides may result in secondary repellence due to the presence of cadavers near a treated area (Henderson et al. 2016, Chouvenc 2018). Imidacloprid and chlorantraniliprole are also nonrepellent chemicals and cause delayed mortality, providing some time for horizontal transfer of the a.i. among termite nestmates. However, secondary repellence might be a problem for transferring toxin (Yeoh and Lee 2007, Henderson et al. 2016). Bifenthrin, is a repellent insecticide that prevents foraging of termites in the treated area and thus maintains a barrier against attack by *C. formosanus* (Yeoh et al. 2006).

Effective and persistent termiticides with low mammalian toxicity are desired for termite control. However, the efficacies of soil termiticides are limited by substrate composition and termiticidal properties (Su and Scheffrahn 1998). Persistence and degradation of a termiticide in soil is dependent on factors such as soil type, moisture, organic matter content, pH, type of termiticide, and rate of initial termiticide application (Forschler and Townsend 1996, Racke et al. 1996, Saran and Kamble 2008). For example, when formulated termiticides are applied to soil at lower concentrations, bioavailability to termites may be reduced, particularly in soils with high clay content, organic matter content, and pH (Henderson et al. 1998). In addition, sorption coefficient (Koc), Kow, water solubility of a.i., and hydrolysis may also influence the persistence of a.i. in the environment. The values of water solubility, Koc, Kow, and hydrolysis half-life of some of the a.i. used in this study are summarized in Table 1.

In addition to soil and chemical properties, flooding of soils is another important factor affecting the efficacies of soil termiticides. Flooding may induce leaching and thereby reduce the concentration of chemicals present in the substrate. Similarly, hydrolysis of active ingredients can also result from flooding, which may affect its efficacy in treated soil. The hydrolysis of fipronil, imidacloprid, and chlorantraniliprole is high in basic conditions but stable in acidic and neutral conditions (Bobé et al. 1998, Zheng and Liu 1999, Bentley et al. 2010). A study by Smith and Rust (1992) suggests that water can play a role in the movement of chemicals away from a treated area and may reduce the efficacy of chemicals. Retention and loss of chemicals after flooding is affected by soil carbon content (Shuai et al. 2012). Toxicity from fipronil and bifenthrin on treated substrates was affected by moisture level (Mohapatra and Ahuja 2009, 2010). Shuai et al. (2012) reported a loss in toxicity in fipronil-treated soil, dependent on soil carbon content in simulated rainfall conditions. Similarly, the rate of degradation of imidacloprid in treated soil increased with an increase in moisture content (Mahapatra et al. 2017). Keefer and Gold (2014b) reported a total loss of imidacloprid from treated soil after field-simulated leaching. However, in a laboratory study, concentrations of imidacloprid and bifenthrin when applied at termiticidal application rates were found to be unaffected by varying moisture levels in treated soil and bedding materials over 24 mo in Australia (Baskaran et al. 1999). Regarding bifenthrin, Mohapatra and Ahuja (2009) stated that its degradation is also independent of the type of substrate because it appears to bind strongly to soil.

Considering the water solubility of active ingredients present in formulated termiticides and the prevalence in some areas of poor water drainage systems where rainwater may remain longer than a day, the efficacies of commonly used termiticide formulations under flooding need further study. Although studies on degradation and leaching of termiticides from treated soils have been conducted, no published information is available on simulated flooding conditions that last for a week on poorly drained termiticide-treated soils. Therefore, we conducted a laboratory experiment to investigate the possible loss of termiticidal activity following a week of simulated flooding. For this study, we evaluated the effects of flooding on the efficacy of four commonly used termiticides by 1) measuring percentage loss of the a.i. in flooded substrates and 2) quantifying the mortality of termites in substrates previously exposed to flooded or unflooded conditions.

## Materials and Methods

### Termiticides

Termidor (BASF Corp., Florham Park, NJ), Altriset (DuPont Corp., Wilmington, DE), Premise (Bayer Corp., Pittsburgh, PA), and Talstar Pro (FMC Corp., Philadelphia, PA) were used in the experiment. All formulated insecticides were provided by their manufacturers. The active ingredients (a.i.) in Termidor, Altriset, and Premise are fipronil, chlorantraniliprole, and imidacloprid, respectively. These termiticides are considered nonrepellent termiticides. The a.i. in Talstar Pro is bifenthrin, a repellent termiticide.

### Termites

Groups of termites from two colonies of *C. formosanus* were used in this experiment. Both groups were collected from Brechtel Memorial Park, New Orleans, LA, one in 2013 and another in 2017, using the milk crate trap technique (Gautam and Henderson 2011b). Colonies were maintained at Louisiana State University, Baton Rouge, LA, in 140-liter trashcans at 25–28°C on wet wood until the experiment was conducted.

### Substrates

Sand and soil were used as substrates for this study. Sand was fine-grade masonry (Louisiana Cement Products, LLC, Greenwell Springs, LA) purchased from a hardware store. Soil was collected from Brechtel Memorial Park, New Orleans, LA. The soil was sent to Soil Testing and Plant Analysis Lab in the School of Plant, Soil, and Environmental Sciences of the LSU AgCenter (Baton Rouge, LA) for analysis. The analyzed soil was clay soil of Westwego series with an organic matter content of 5.49%, total C of 3.851%, N content of 0.346%, and pH 5.10. Both substrates were autoclaved (12 cycles at 250 K for 60 min) and then placed in an incubator at 60°C for 24 h for drying.

### Treatment of Substrates

The experimental procedure used was intended to simulate field conditions in which flooding of soil does not occur immediately after

**Table 1.** Summarized value for water solubility, Koc, log Kow, and hydrolysis half time of the active ingredients used

| Active ingredients | Water solubility (mg/liter) | Organic carbon normalized partition coefficient (Koc) | Octanol–water partition coefficient (logKow) | Hydrolysis half time |
|---|---|---|---|---|
| Fipronil | 1.9–2.4[a] | Av = 825 ± 214[a] | 3.50[a] | >100[a] |
| Imidacloprid | 610[b] | | 0.57[b] | |
| Bifenthrin | 0.1[c] | Log (Koc) = 5.4[c] | 6[c] | >30[c] |
| Chlorantraniliprole | 0.9[d] | Log (Koc) = 2.6[d] | 2.9[d] | |

[a]Data cited from Gunasekara et al. (2007).
[b]Data cited from Bonmatin et al. (2015).
[c]Data cited from Oros and Werner (2005).
[d]Data cited from Vela et al. (2017).

termiticide application. The amount of substrate (soil or sand) to be treated and the amount of water needed for the flooding treatment were determined in the laboratory. Termiticide concentrations used in this experiment were chosen to simulate levels of residual termiticide present in the soil at various time points after application, i.e., rates of termiticides used were lower than label rates to simulate degradation of termiticides over time. For example, the labeled rate of Termidor is 23.66 ml in 3.785 liters of water (≈0.62% Termidor in Termidor–water mixture resulting into 0.0564% fipronil in total mixture) for perimeter treatments, but rates lower than the label rate were used in this experiment to simulate reduced termiticide concentrations over time. A 20% moisture level by weight of soil was used for the clay (soil) substrate, and a 10% moisture level by weight of sand was used for sand. The moisture levels in this experiment were prepared and maintained as in Bhatta et al. (2016). Amounts of termiticides required to attain the desired concentrations were calculated based on wet substrate weight. For example, to attain rates of 1, 10, and 25 ppm fipronil with a 20% moisture level in soil, 6.59, 65.9, or 164.9 µl of Termidor (a.i. fipronil 9.1%) was added to each of 100-ml deionized water. Water and termiticide were mixed and added to 500 g of soil in three Ziploc bags (S. C. Johnson and Son, Racine, WI). Using the same product and concentrations with 10% moisture in sand, 6.04, 60.5, or 151.1 µl of Termidor were added to 50 ml of deionized water. The water–termiticide mixture was then added to 500 g of sand in three different Ziploc bags. The treated substrates were mixed by hand in sealed Ziploc bags for approximately 5–10 min to distribute the insecticide evenly throughout the substrate. About 200 g of Termidor-treated soil and 250 g of Termidor-treated sand were saved for further experiments and rest was properly discarded. Identical procedures were used to treat sand and soil substrates with imidacloprid, bifenthrin, and chlorantraniliprole at 1, 10, and 25 ppm. Six separate Ziploc bags were prepared for each of these insecticides, three for soil and three for sand.

For the untreated control substrates used in the experiment for reference, deionized water and substrates were mixed gently in sealed Ziploc bags by hand in a manner identical to that used for the insecticide-treated substrates. Six Ziploc bags, three for sand, and three for soil were prepared for controls. The percentage of moisture in the controls was the same as for the treated substrates.

After the substrates were gently mixed with water–termiticide mixtures at different concentrations in sealed Ziploc bags, they were opened and kept under fume hood for drying for 2 d. Substrates were redistributed periodically to ensure uniform drying. The visibly dry substrates obtained were used for experiments without further processing. Thirty grams (5 g in each of six Petri dishes) of dried substrates were used to set up the no-choice bioassays for unflooded substrates, whereas 175 g of sand and 125 g of soil were subjected to flooding, and 25 g of each treated substrate was taken for chemical analysis.

## Flooding Treatment

Termiticide-treated and -untreated (control) substrates were subjected to simulated flooding treatments. Flooding was simulated using 9.6-cm-tall plastic cups (Better Living Brands, LLC, Pleasanton, CA). Twenty-one small holes were made in the bottoms of cups using a sewing needle of 1.1 mm diameter. Holes were equidistant from one another at 5.4 mm and were placed in the center of the bottoms of the cups (3.6 cm diameter) to allow for water drainage. Bottoms of the cups were filled to a height of 2.7 cm with clean and washed TERM Particle Barrier (TERM Particle Barrier, Polyguard, Ennis, TX) to facilitate flow of water. The next 4.2-cm-deep cup section was filled with either sand (175 g) or soil (125 g), and the remaining 2.7 cm was allocated for the addition of deionized water to the cup. After each cup was filled with TERM Particle Barrier followed by substrates, it was placed inside of a second cup (with no holes) to retain the floodwater. Then 130-ml deionized water was added to the cups containing treated substrates. After 1 h, the bottom cup was removed, and water was allowed to drain completely. Drained water was collected in a waste container and sealed for disposal. Draining of water after 1 h was intended to simulate rainfall percolating through soil and to remove termiticide trapped in bottom cup. A second time, the substrate cups were placed in the cups without holes, and 90 ml of deionized water (for cups with sand) or 70 ml (for cups with soil) was added. This water was retained for 1 wk. The cups with the treated substrates were not disturbed until day 7, when the water was drained as described previously and substrates (sand and soil at 1, 10, and 25 ppm) were collected individually. For the collection of substrates, cups were cut using scissors and the lump of wet substrate was removed and placed in open plastic containers for drying under a fume hood. Our objective was to study the effect of residual termiticide present on termiticide-treated soil (if present after a flood) on termite mortality; therefore, a.i. concentrations were not measured in drained water.

The procedure for flooding untreated controls was identical to that described above except untreated substrates were used in the cups. Thirty cups were used to simulate flooding in the laboratory setting: six cups were for each of four termiticides, three for sand and three for soil, with one cup for each concentration, and six cups for control, three for sand and three for soil.

The substrates collected after flooding were kept under a fume hood for 4 d to dry. On the fifth day, the dried sand and dried soil were ground into fine powder using a clean mortar and pestle. For each treated sample 25 g of substrate for each concentration was extracted by the Department of Agricultural Chemistry LSU Agricultural Center (Agricultural Chemistry Lab, 12 Ag, Baton Rouge, LA). An additional 30 g (5 g in each of six Petri dishes) of substrate was used to conduct a no-choice bioassay.

## Chemical Analysis

Methods used for chemical analyses were adapted from Lanka et al. (2014) and Adeniyi et al. (2016). For extraction of chemicals from treated substrates, samples were prepared by drying on a sheet of aluminum foil under a fume hood. Following drying, 25 g of substrate was weighed and added to a 250-ml flask with 100 ml of solvent. Ethyl acetate was used as a solvent for fipronil and bifenthrin, and acetonitrile was used for imidacloprid and chlorantraniliprole. Each flask was agitated overnight on a mechanical shaker. The resulting solution was filtered through filter paper with a Buchner funnel. The extracted solution was rinsed with solvent three times and transferred into a 200-ml concentrator tube. The volume was reduced on a TurboVap at 40–50°C under nitrogen to 2–3 ml. The concentrated solution was dried in filter paper with anhydrous sodium sulfate followed by three solvent rinses and transferred to a 15-ml centrifuge tube. Analyses of fipronil and bifenthrin concentrations were carried out by gas chromatograph–mass spectrometer (GC–MS), whereas analyses of imidacloprid and chlorantraniliprole concentrations were carried out by liquid chromatography–mass spectrometry (LC–MS).

## GC/MS Analysis (Fipronil and Bifenthrin)

An Agilent 6890 GC interfaced with an Agilent 5973 quadrupole MS was used for the analysis of fipronil and bifenthrin. An Agilent 7683 series autosampler was used to inject sample extracts and standards onto a 30-m Restek 5MS GC column with internal diameter of 0.25 mm and film thickness of 0.25 µm. Instrument control and quantitative data analyses were carried out in Agilent Chemstation software (Agilent, Santa Clara, CA). Injection volume of the extracts was 2.0 µl with pulsed splitless injection at 20 psi for 0.74 min. The injector temperature was 250°C, and transfer line temperature was 280°C. The carrier gas in the line was helium with the constant flow rate of 1.2 ml/min. The MS was operated in electron impact ionization mode with the MS ion source at 230°C and quadrupoles at 150°C. The electron multiplier was set 200 V above the PFTBA-autotuned setting. For screening and quantitative analysis, selected ion monitoring mode was used. For initial identification of pesticide, detection of the characteristic ion peaks and their relative abundances (%) and the comparison of retention times with those observed in the analytical standard were used. The average recovery rates of fipronil from sand and soil were 77 and 83%, whereas the average recovery rates of bifenthrin from sand and soil were 85.6 and 87.2%, respectively.

### LC/MS/MS Analysis (Imidacloprid and Chlorantraniliprole)

For sample analysis of imidacloprid and chlorantraniliprole, a Waters UPLC Aquity liquid chromatograph interfaced with a Waters TQD triple quadrupole MS/MS was used. Two different injection rates for imidacloprid and chlorantraniliprole were required.

For imidacloprid, the injection volume of extract was 10 µl in water with 0.1% formic acid as mobile phase A and acetonitrile with 0.1% formic acid as mobile phase B. A flow rate of 0.3 ml/min was used in the beginning at 98%A/2%B, changing to 2%A/98%B over 8 min. Thereafter, conditions were changed back to 98%A/2%B over 0.5 min, and these conditions were maintained for 12 min. For chlorantraniliprole, the injection volume of extract was 10 µl in water with 0.1% formic acid as mobile phase A and acetonitrile with 0.1% formic acid as mobile phase B. Flow rate of 0.3 ml/min was used in the beginning at 95%A/5%B changing to 65%A/35%B over 2 min. These conditions were held at 65%A/35%B for 1 min and changed to 5%A/95%B over 1 min and fully changed to original condition and equilibration for 7 min.

The triple quadrupole operated in electrospray positive mode with capillary at 3.84 kV and extractor at 3.66 V for imidacloprid and 2.0 V for chlorantraniliprole. Source temperature at 120°C, desolvation temperature of 400°C, and nitrogen flow of 500 liter/h were maintained for both chemicals. The collision gas used was argon with the flow of 0.18 ml/min. For comparison of ion peaks and their relative abundances as well as comparison of retention time with those observed in the analytical standard, multireaction monitoring was used. The average recovery rates of imidacloprid from sand and soil were 85 and 93%, whereas the average recovery rates of chlorantraniliprole from sand and soil were 93.75 and 86%, respectively.

### No-Choice Bioassays

No-choice bioassays were conducted using flooded and unflooded, termiticide-treated, and untreated substrates. There were 312 total dishes (two flooding treatments × four insecticides × three concentrations × three replicates × two colonies × two substrates, plus control dishes). These dishes consisted of 144 treated and unflooded, 144 treated and flooded, and 24 controls, with controls comprising 12 untreated and unflooded, and 12 untreated and flooded. For soil, 5g of substrate in each of 156 Petri dishes were used for bioassays. Filter paper (Ahlstrom qualitative filter paper, grade 615, diameter 7.5 cm, Ahlstrom, Helsinki, Finland) was placed in the bottom of each Petri dish followed by 5-g soil to which 1 ml of deionized water was added (20% wt of 5-g soil = 1-ml deionized water).

The procedure used for sand was similar to that used for soil except 0.5 ml (10% wt:wt) water was used to wet the sand. Similarly, for controls, 5 g of untreated substrates were used in the Petri dishes while keeping the moisture level the same as in the treated substrates. After placing filter paper and wetted substrates into dishes, 31 termites (20 workers and 11 soldiers) were added, and the Petri dishes were sealed using Parafilm (Bemis flexible packaging, Neenah, WI) to reduce the moisture loss and exclude contaminants. Readings were taken by observing the termites without opening the Petri dishes. Mortality of termites was recorded daily through day 6. Petri dishes were disturbed slightly from outside, and if termites did not move for 5 s, they were scored as dead.

### Statistical Analysis

For the no-choice bioassays on day 6, a three-way ANOVA was used to test the effect of flooding on mortality of termites, followed by Tukey means comparisons for mortality of each treatment combination. The three independent variables in the analysis were insecticide, dose, and flooding. Two separate analyses for sand and soil were conducted because we did not aim to statistically compare the impact of flooding between sand and soil treatments. Due to time and resource constraints, only a single sample was sent for chemical analysis of each termiticide at different concentrations and substrates; therefore, statistical analysis could not be performed.

## Results

### Chemical Analysis

The reduction in concentrations of chemicals in substrates subjected to flooding relative to substrates not subjected to flooding was greater from sand than from soil for all the tested chemicals (Table 2). Imidacloprid was the most leachable insecticide, and bifenthrin the least leachable insecticide. Losses of fipronil and chlorantraniliprole were intermediate to imidacloprid and bifenthrin. Reductions were greatest from the 1 ppm concentrations for all insecticide/substrate combinations except bifenthrin and imidacloprid in soil. The concentration of bifenthrin at the 1 ppm rate after flooding in soil was 0.2 ppm higher than the concentration in soil under unflooded conditions. The slight increase in concentration is attributable to lack of replications in chemical analysis. Also, reduction in bifenthrin at 10 ppm was greater from soil than from sand, which was different than the general trend observed for greater loss from sand than from soil.

### No-Choice Bioassays

Mortality of *C. formosanus* was assessed on day 6 as cumulative mortality for all treatment combinations. Mortality of *C. formosanus* in control treatments, which consisted of flooded and unflooded sand and soil not treated with insecticides, was less than 5% in both flooded and unflooded substrates. The effect of the three-way interaction of insecticide, flooding, and dose on mortality of *C. formosanus* was statistically significant in treated sand ($F = 28.49$; df = 6,130; $P < 0.0001$) but was not statistically significant in treated soil ($F = 0.26$; df = 6,130; $P = 0.9531$). In sand, all two-way interactions among the main effects of insecticide, dose, and flooding were significant (Table 3a). The main effect of flooding, termiticides, and dose on mortality of *C. formosanus* was

**Table 2.** Concentrations in parts per million of fipronil, imidacloprid, bifenthrin, or chlorantraniliprole in soil and sand samples subjected or not subjected to 1 wk of simulated flooding

| Chemical | Substrates | Unflooded sample concentration (in ppm) | Flooded sample concentration (in ppm) | Difference in concentration (%) |
|---|---|---|---|---|
| Fipronil 1 ppm | Sand | 1.06 | 0.0052 | −99.50943 |
| Fipronil 10 ppm | Sand | 8.78 | 3.62 | −58.76993 |
| Fipronil 25 ppm | Sand | 27.69 | 10.1 | −63.52474 |
| Fipronil 1 ppm | Soil | 1.042 | 0.67 | −35.70058 |
| Fipronil 10 ppm | Soil | 8.237 | 6.55 | −20.48076 |
| Fipronil 25 ppm | Soil | 21.26 | 17.52 | −17.59172 |
| Imidacloprid 1 ppm | Sand | 1.3 | 0.0034 | −99.73846 |
| Imidacloprid 10 ppm | Sand | 12.58 | 0.031 | −99.75358 |
| Imidacloprid 25 ppm | Sand | 26.16 | 0.12 | −99.54128 |
| Imidacloprid 1 ppm | Soil | 0.997 | 0.459 | −53.96189 |
| Imidacloprid 10 ppm | Soil | 7.81 | 4.07 | −47.88732 |
| Imidacloprid 25 ppm | Soil | 29.72 | 12.45 | −58.10902 |
| Bifenthrin 1 ppm | Sand | 1.68 | 1.388 | −17.38095 |
| Bifenthrin 10 ppm | Sand | 7.84 | 7.05 | −10.07653 |
| Bifenthrin 25 ppm | Sand | 29.41 | 25.94 | −11.79871 |
| Bifenthrin 1 ppm | Soil | 1.03 | 1.236 | +20 |
| Bifenthrin 10 ppm | Soil | 7.56 | 5.11 | −32.40741 |
| Bifenthrin 25 ppm | Soil | 20.59 | 19.83 | −3.691112 |
| Chlorantraniliprole 1 ppm | Sand | 3.304 | 1.925 | −41.73729 |
| Chlorantraniliprole 10 ppm | Sand | 16.542 | 13.82 | −16.45508 |
| Chlorantraniliprole 25 ppm | Sand | 36.81 | 23.09 | −37.27248 |
| Chlorantraniliprole 1 ppm | Soil | 3.41 | 2.44 | −28.44575 |
| Chlorantraniliprole 10 ppm | Soil | 13.84 | 13.47 | −2.67341 |
| Chlorantraniliprole 25 ppm | Soil | 26.09 | 18.85 | −27.7501 |

'−' in the 'Difference in concentration' column indicates a lower concentration of chemical in substrates subjected to flooding; '+' indicates higher concentration of chemical in substrates subjected to flooding.

**Table 3.** Three-way ANOVA for mortality of termites in termiticide-treated unflooded or flooded sand (a) and soil (b)

(a) Type 3 tests of fixed effects

| Effect | Numerator df | Denominator df | *F* value | Pr > *F* |
|---|---|---|---|---|
| Insecticide (I) | 3 | 130 | 297.88 | <0.0001 |
| Dose (D) | 2 | 130 | 179.29 | <0.0001 |
| I × D | 6 | 130 | 28.88 | <0.0001 |
| Flooding (F) | 1 | 130 | 65.11 | <0.0001 |
| I × F | 3 | 130 | 19.24 | <0.0001 |
| F × D | 2 | 130 | 12.99 | <0.0001 |
| I × F × D | 6 | 130 | 28.49 | <0.0001 |

(b) Type 3 tests of fixed effects (in soil)

| Effect | Numerator df | Denominator df | *F* value | Pr > *F* |
|---|---|---|---|---|
| Insecticides (I) | 3 | 130 | 56.64 | <0.0001 |
| Dose (D) | 2 | 130 | 59.95 | <0.0001 |
| I × D | 6 | 130 | 4.69 | 0.0002 |
| Flooding (F) | 1 | 130 | 0.24 | 0.6252 |
| I × F | 3 | 130 | 1.57 | 0.1988 |
| F × D | 2 | 130 | 1.42 | 0.2449 |
| I × F × D | 6 | 130 | 0.26 | 0.9531 |

also statistically significant (Table 3a). However, in soil, the three-way interaction and all the two-way interactions involving the main effect of flooding were not statistically significant at 0.05 level of significance (Table 3b).

Based on the Tukey analysis, a highly significant difference in mortality of *C. formosanus* under flooded and unflooded conditions was observed in fipronil-treated sand at 1 ppm ($P$ < 0.0001). However, no significant differences in mortalities between flooded and unflooded conditions were observed at the 10 or 25 ppm concentrations (Fig. 1a). Mortalities of *C. formosanus* in imidacloprid treatments differed significantly between flooded and unflooded sand treated with 10 ppm ($P$ < 0.0001) and 25 ppm ($P$ < 0.0001), but the effect was not significant at 1 ppm (Fig. 1b). Flooding did not have significant effects on mortalities of *C. formosanus* in bifenthrin-treated sand at any concentrations tested (Fig. 1c). Flooded and unflooded sand showed significant differences in mortalities of *C. formosanus* at 1 ppm for chlorantraniliprole ($P$ < 0.0001). However, mortalities did not differ significantly between flooding treatments at 10 or 25 ppm in chlorantraniliprole-treated sand. In soil treated with all four termiticides, mortalities under flooded and unflooded conditions did not differ significantly. However, slight numerical increases in mortalities in flooded conditions were observed in soil treated with imidacloprid and chlorantraniliprole at 10 and 25 ppm, respectively (Fig. 1b and d), whereas some fungal blooms were observed in some of the replicates in flooded soil treated with imidacloprid.

Based on the mortalities observed in no-choice bioassays, in sand bifenthrin was found to be the most effective chemical after a flood, followed by fipronil and chlorantraniliprole, whereas imidacloprid was least effective after a flood. In soil, bifenthrin and fipronil were similar in effectiveness followed by imidacloprid. Chlorantraniliprole was the least effective in soil. In addition, the effectiveness of all insecticides increased as the concentration of insecticide increased.

## Discussion

Although the lack of replication of the chemical analyses limits the inferences that can be made about leaching of termiticides after flooding, the chemical analysis of the soils and sands suggest that all termiticides used in this experiment leached to some extent and that the leaching was greater in sand than soil (Table 2). Although Keefer and Gold (2014a) reported that fipronil exhibits low potential for leaching from the soil profile, the concentration of fipronil was reduced after a simulated flood in our experiment. Shuai et al. (2012), under simulated rainfall conditions, observed that the leaching potential of fipronil from soil was inversely related to soil organic carbon content. They observed 29% concentration loss in 24 h of simulated rainfall from soil. The lower average loss of fipronil in our study compared with the study by Shuai et al. (2012) could be due to the differences in type of soil and their carbon content. The carbon content of the soil used in our experiment was higher (3.851%). Imidacloprid is a water-soluble chemical (Keefer and Gold 2014b); however, large concentration losses were observed only in sand (Table 2). The leaching and retention of imidacloprid in soil are also dependent on soil texture and organic matter content, i.e., leaching of chemical is higher in sandy soil compared with clay soil while retention is greater in clay soil (Liu et al. 2006, Bajeer et al. 2012, Samnani et al. 2013), which explains the variation in the loss of imidacloprid from sand and soil in our study. In contrast, bifenthrin had the lowest loss after flooding (except at 10 ppm in soil) among the chemicals tested in this experiment, which is consistent with the findings by Baskaran et al. (1999). In fact, an increase of 0.2 ppm after flooding was observed in soil treated with 1 ppm of bifenthrin, a result that is probably attributable to an uneven distribution of chemical in the substrate prior to flood and to the lack of replication. Chlorantraniliprole also leached to some extent from both substrates (Table 2). Similar to our results, Vela et al. (2017) reported that chlorantraniliprole is leachable. The adsorption capacity of a substrate is dependent on the organic matter content (Paszko 2006), and the performance of a termiticide is highly influenced by soil type and organic matter content (Smith and Rust 1993), which probably accounts for the observed variations in reductions in concentrations of termiticides on substrates after simulated flood.

Because our bioassays lasted for more than 24 h, we provided termites with deionized water on filter paper as a food source, but

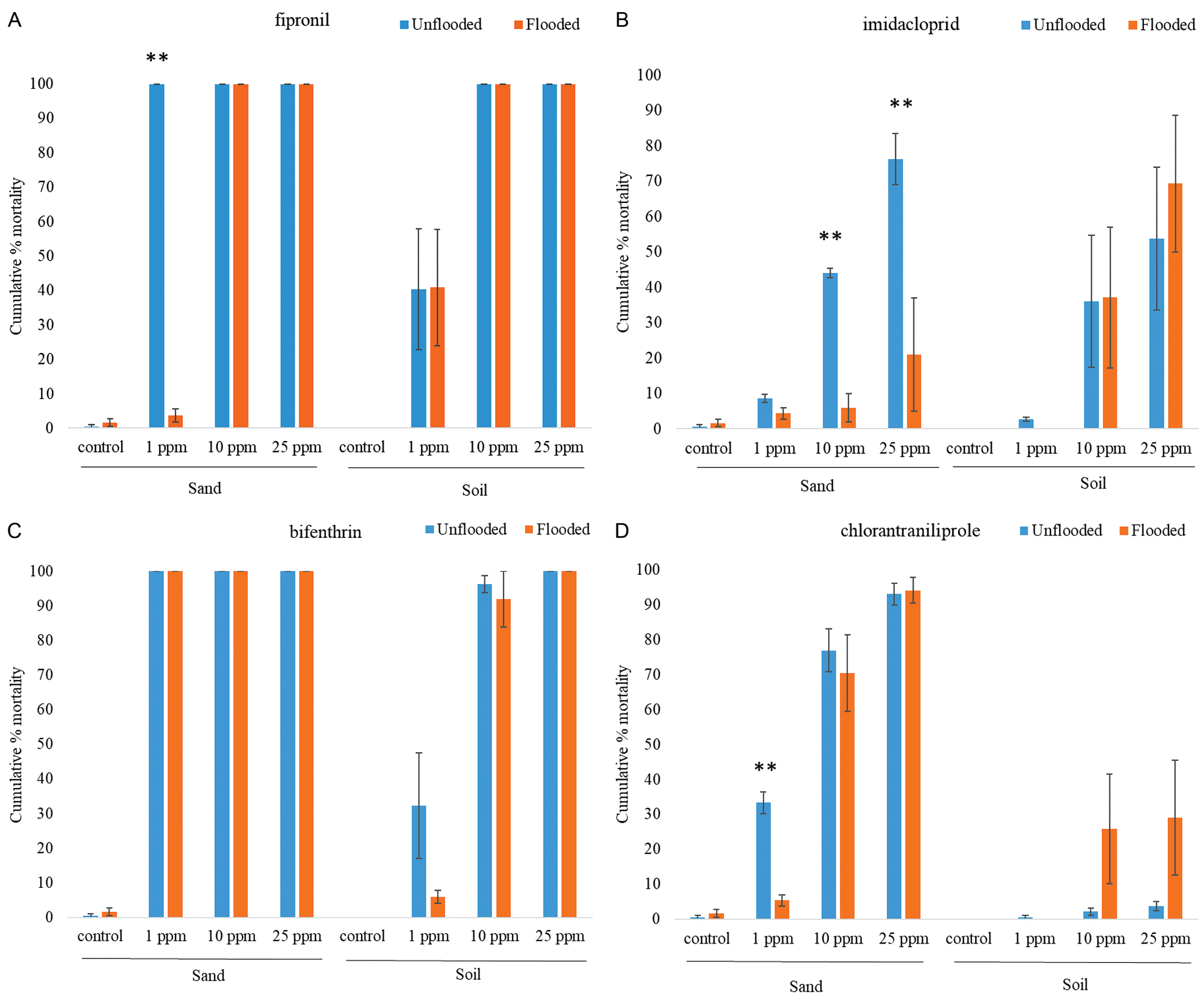


**Fig. 1.** (a–d) Cumulative mean percent mortalities (±SEM) of *Coptotermes formosanus* in termiticide-treated or untreated, unflooded, or flooded sand and soil on day 6. Means were compared using Tukey means comparisons procedure. **Mortality is significantly different between flooded and unflooded substrates ($P = 0.001$).

the percentage of deionized water used was different for sand and soil. The study by Baskaran et al. (1999) supports the use of two different moisture levels in sand and soil to obtain similar levels of free water in the substrates without affecting the rate of degradation of termiticides. Lys and Leuthold (1994) reported that, at moisture levels of 9.5% in sandy soil and 15.4–25.1% in clay soil, workers of *Macrotermes subhyalinus* Rambur (Isoptera: termitidae) and *Macrotermes bellicosus* Smeathman (Isoptera: termitidae) can obtain free water.

In no-choice bioassays, fipronil was still effective after a flood at higher concentrations irrespective of the substrate but at 1 ppm fipronil was no longer effective. Fipronil was found effective at as low as 0.5 ppm in sand against Western subterranean termites *Reticulitermes hesperus* Banks (Blattodea: Rhinotermitidae) (Saran and Rust 2007), which is similar to our finding where termite mortality was effective before flooding at 1 ppm fipronil-treated sand (Fig. 1a). However, the persistence of fipronil in substrates increases with increase in initial concentration applied to soil, organic matter, and clay content in soil (Bobé et al. 1997, Saran and Kamble 2008). Therefore, soil with higher organic matter content when treated with fipronil-based termiticides can provide longer protection against *C. formosanus* compared with sandy soil and is justified by the results from no-choice bioassays and chemical analysis in this study. In contrast to fipronil, treatment with imidacloprid in sand was no longer effective after flooding at any concentrations tested, suggesting that imidacloprid was washed away with the flood water and was unable to provide toxic effect to *C. formosanus*. This inference is supported by the chemical analysis data on Table 2. Similarly, Keefer and Gold (2014b) reported that imidacloprid was completely leached from treated soil in 6 mo. Slight increases in mortality in soil at higher concentrations could be due to saprophytic fungal blooms, which may occur in loam soil treated with imidacloprid (Ramakrishnan et al. 1999) or due to better distribution of the active ingredients in soil by floodwater. In bifenthrin treatments, effective mortality of *C. formosanus* was observed irrespective of the substrates and flooding treatment. In a study by Saran and Kamble (2008), rapid and effective mortality of workers of *Reticulitermes flavipes* Kollar (Blattodea: Rhinotermitidae) with bifenthrin was observed in a continuous exposure bioassay. Rapid mortality of *C. formosanus* in both flooded and unflooded sand treated with higher concentrations of bifenthrin and fipronil was observed in this experiment (100% mortality was observed by day 4), suggesting the persistent nature of bifenthrin and fipronil. Similarly, Smith and Rust (1990) observed higher mortality in bifenthrin-treated soil compared with soil treated with several other pyrethroids, suggesting the greater inherent toxicity of bifenthrin. The inherent toxicity of bifenthrin was least affected by flooding in this experiment.

Chlorantraniliprole treatment also caused effective mortality after a flood at 10 and 25 ppm tested in sand, but the mortality was not effective at the lowest concentration treated. Flooding did not have an apparent effect on the mortality of *C. formosanus* in soil with chlorantraniliprole, but the toxicity was reduced in soil, which could be due to the presence of organic matter, that may reduce the bioavailability of chlorantraniliprole to termites even at label rates (Spomer et al. 2009, Gautam and Henderson 2011a). Like chlorantraniliprole, chlorpyrifos, fenvalerate, cypermethrin, and permethrin demonstrated greater toxicity in sand compared with the soil in continuous exposure bioassays (Forschler and Townsend 1996). Another study by Mao et al. (2011) observed the effectiveness of chlorantraniliprole and cyantraniliprole even at 1.28 ppm on treated sand. In soil with chlorantraniliprole, slight but not significant increases in percent mortality were observed in flooded soil at higher concentrations. We hypothesize that this increase is attributable to better distribution of active ingredients due to floodwater, making the active ingredient readily available to *C. formosanus*. Similarity in the mortalities of *C. formosanus* in flooded and unflooded substrates suggests the persistent nature of chlorantraniliprole.

From a practical standpoint, it appears that the decision of whether or not to retreat after a flood depends on the properties of the active ingredient used, including its inherent toxicity, concentration, its water solubility, Koc, and soil type. Most importantly, the water solubility of formulated insecticides needs to be considered before using in flood-prone areas. Therefore, the areas that have been treated with water-soluble chemicals such as imidacloprid may require a retreatment with less water-soluble chemicals or baits after a flood.

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