The Effect of Foraging Tunnel Treatment with Termidor® DRY on *Reticulitermes flavipes* (Isoptera: Rhinotermitidae)[1]

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ABSTRACT The efficacy of a dry, ready-to-use (RTU) termiticide formulation of fipronil was evaluated against termite foraging activity and survival by injection into foraging tunnels of *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) in a laboratory study. Groups of workers (older than 3rd instars) were placed in bioassay units consisting of a group site and a feeding site connected by termite tunnel(s) within a soil-filled Tygon tube. Two treatments (0.15 and 0.30 mg a.i. fipronil per treatment) were conducted by injecting the doses into a foraging tunnel near the feeding site. Bioassay units were monitored for termite movement utilizing Termatrac® T3i until no movement was detected. Termite movement ceased at day 5 and 7 for the 0.30 and 0.15 mg dose treatments, respectively. Dissection of the bioassay units confirmed 100% mortality at the days when both visual observations and Termatrac T3i indicated no termite movement. Termites in the treated units constructed significantly fewer tunnels post-treatment compared to control termites. Our results provided strong evidence for the efficacy of the dry RTU fipronil formulation against *R. flavipes* activity at the group level when a single tunnel was treated.

KEY WORDS eastern subterranean termites, dry RTU fipronil formulation, control, Termatrac®

Subterranean termites (Isoptera: Rhinotermitidae) forage for cellulosic food sources and frequently cause significant damage to structural wood and wood products. In the United States, Rhinotermitidae, the most economically important family, has an economic value of $3 billion per year (Su 2002), $1 billion of which is attributable to the eastern subterranean termite (Potter 2004) that has a wide geographical distribution (Wang et al. 2009).

Subterranean termites live underground. Their cryptobiotic or "hidden" lifestyle makes their presence and damage difficult to detect. Termites often build tube-like extensions not only underground but also aboveground to connect colonies to food and water sources. These conduits, commonly called mud tubes, become obvious when they extend over concrete foundations and other exposed surfaces and become a valuable sign for detection.

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The control of structural infestations of subterranean termites currently depends on the use of soil-applied termiticides for prevention and remedial treatment (Hu 2011). Soil-applied termiticides are used to treat soil around and beneath the foundation of structures to establish a chemical barrier against termites (Su & Scheffrahn 1990).

Fipronil is one of the most commonly used nonrepellent compounds for termite management in the United States (Hu 2011). Fipronil was identified in 1987 by the French company Rhône-Poulenc and registered in the United States in 1996 as a broad spectrum insecticide in the phenyl pyrazole class (Rhône-Poulenc 1995). Fipronil acts on the insect's central nervous system by blocking the GABA (λ-aminobutyric acid) receptor and regulating chloride channels, resulting in excess neuronal stimulation and eventual death of the exposed insect (Cole et al. 1993). Numerous studies have evaluated the efficacy fipronil as a liquid soil termiticide. The effect of fipronil in reducing the foraging range and activity of subterranean termites has been investigated in both laboratory (Saran & Rust 2007, Quarcoo et al. 2010, 2012) and field studies (Potter & Hillery 2002, Kard 2003, Hu & Hickman 2006). The nonrepellency and horizontal transmission from worker to worker, and worker to soldier, have been studied using groups of termites (Hu 2005, Hu et al. 2006, Saran & Rust 2007). Hu et al. (2006) further demonstrated vertical transmission from foraging workers to reproductives as well as horizontal transmission to other caste members in laboratory colonies. The influences of liquid fipronil concentration and dose, duration of exposure, and the ratio of donor to recipient termites have been examined for various termite species (Ibrahim et al. 2003, Shelton & Grace 2003, Remmen & Su 2005). The impact of soil type, compaction, temperature gradients, pH value, and the presence of various woods on the penetration and action of fipronil in the soil has also been examined (Mulrooney et al. 2007, Gautam & Henderson 2011).

Recently, a new formulation of dry ready-to-use (RTU) fipronil has been developed. This product can be placed directly into aboveground foraging tubes or other matrices where termites are active. The purpose of this study was to determine the efficacy of tunnel treatments at various doses of this formulation against the foraging and survival of eastern subterranean termites, *Reticulitermes flavipes*.

Materials and Methods

Termites. Eastern subterranean termites were collected from field colonies in the city of Auburn, Alabama (Lee County) using underground traps as described by Hu & Appel (2004). Traps consisted of open-bottom plastic buckets (18 cm high, 13 cm in diameter) provisioned with corrugated cardboard rolls (15 cm high and 11 cm in diameter) that were set in the ground. Closed-bottom plastic buckets (18 cm high, 13 cm internal diameter) were used to transfer corrugated cardboard rolls with termites to the laboratory. Termites were extracted by gently tapping the cardboard to allow termites to drop onto a moist paper towel. Workers and soldiers were used within two days of collection.

Soil. Soil was obtained from an area immediately surrounding a termite colony in a field in Auburn. The soil was sieved to remove debris and sterilized in an autoclave (Thermo Fisher Scientific, Waltham, MA) at 60°C for 24 h, two days prior to the experiment. Distilled water was added to the soil (8 ml H_2O/100 g of soil). Moistened soil was then used to fill Tygon® (VWR Corp., Radnor, PA) tubes (0.8 cm inside diameter) and plastic cylinders.

Chemical. Termidor® DRY, a RTU dry formulation containing 0.5% active ingredient fipronil [5-amino-1-(2,6-dichloro-4-(trifluoromethyl) phenyl)-4-((1,R,S)-(trifluoromethyl)sulfinyl)-1-H-pyrazole-3-carbonitrile] was provided by BASF Corporation (BASF Pest Control Solutions, St. Louis, MO). Blank formulation containing no active ingredient but only the micro-crystalline cellulose carrier, Microllose™, was also provided by BASF Corporation (Research Triangle, NC).

Experimental design. Each bioassay unit was composed of two plastic cylinders (8.1 cm high and 8.5 cm in diameter) connected by a soil-filled Tygon tube (91.4 cm in length). One cylinder was the feeding site, which contained one block ($1.8 \times 1.8 \times 2.1$ cm^3) of damp southern yellow pine as a food source. The other cylinder was the group site with a yellow pine block on the bottom of the cylinder covered with a 3-cm deep layer of moistened soil. A small hole (1 cm in diameter) was burned into the side at the base of each cylinder using a soldering iron. The two cylinders were connected at the holes with Tygon tubing using hot glue. Groups of 500 termites (2 soldiers per 100 workers, the ratio found in field) were introduced into the group site cylinder. All cylinders were covered with lids and sealed with Parafilm to maintain moisture. Fifteen bioassay units were maintained in the dark at room temperature to allow termites to tunnel from the group sites through the connecting tubes into the feeding sites.

Treatments. The dry RTU 0.5% fipronil formulation was injected into a termite tunnel within the Tygon tube two days after the tunnels were established. Two doses (0.15 and 0.30 mg) of the active ingredient were tested. The blank formulation was used as a control and applied at a volume corresponding to the low rate of the active formulation. Using a soldering iron, a small hole (0.6 mm) was punched in the Tygon tubes 30 cm away from the feeding site and directly into a termite tunnel. The designated doses of RTU fipronil formulation and blank formulation were injected into the holes in the Tygon tubes using a customized injecting device. The injection device consisted of an Eppendorf-style pipet tip (200 μL in volume and 4.8 cm in length) (Fisher Health Care Corp., Houston, TX) attached to a 10-ml syringe tip. The syringe plunger forced air and the dry formulation into the termite tunnel, with the tip of the applicator aimed at the group site at a 45 degree angle, so the treatment could be spread a distance of 8 to 12 cm inside the tunnel without blocking the tunnel at the treatment point. Each treatment was replicated five times.

Data collection. The distances termites tunneled through the moist soil in the Tygon tube from the group site towards the feeding site was visually observed and recorded daily before the treatment. This was to determine the time taken for termites introduced into the group site to tunnel through Tygon tube into feeding site. New tunnel construction within the feeding site arena and termite movement within the bioassay units were recorded every other day after the treatment until no termite movement was detected. New tunnel construction inside the feeding site was noted by visual observations. Termite movement within the bioassay units was recorded using two methods: 1) Visual observations of termites' movement at the bottom of the transparent cylinders and the tunnels at portions of the Tygon tube where no soil/mud had been deposited during tunnel formation; and 2) Termatrac T3i recording (Termatrac Australia Pty Ltd.

Queensland, Australia). The Termatrac T3i was used to corroborate the visual observations. After both visual observation and Termatrac T3i corroboration indicated no movement, the cylinders were disassembled to count living termites. Termites were recorded as dead when they could not move any appendages.

The Termatrac is a 3-in-1 device that comes with a remote thermal sensor with a laser guide, a built-in moisture sensor, and patented termite detection radar. It emits a fixed frequency microwave beam and measures the intensity and frequency of signals reflected back to a collocated receiver (Tirkel et al. 1997, Protec USA 2002). A signal processor computes the intensity of the reflected energy and the difference between emitted and reflected frequencies, and displays the result on a Bluetooth® (Bluetooth Special Interest Group, https://www.bluetooth.org/en-us) wireless PDA (personal digital assistant). Termatrac can detect motion or vibration by any object in the field of view whose dielectric constant (a relative measure of the capability to store electric charge) differs from that of the substrate (Edde 1993, Protec USA 2002). Any moving objects not of interest were excluded from the field of view. Termatrac T3i was positioned under each cylinder (i.e., feeding site and group site) and above the Tygon tube. Termatrac T3i detection radar signals were calibrated to identify termite location and intensity of activity which was measured in gain (y-axis) over time (x-axis), positively correlating gain with movement intensity. The signal graphs were sent to a computer. The peaks below 2 gain, between 2 and 4 gain, and above 4 gain from each graph were counted, and the average of the peaks for each graph was used as responding value.

Statistical analyses. Pre-treatment termite tunneling data and post-treatment termite mortality were analyzed using one-way analysis of variance (ANOVA) utilizing Minitab 13 software (Proc GLM, Minitab Institute, State College, PA) (Minitab Institute 2003).

Results

Behavioral observations pre-treatment. Introduction of termites into the group site cylinders resulted in an initial burst of tunneling activity. Termites radiated out through the moist soil to the base of the cylinders, then tunneled into the Tygon tubes toward the feeding site cylinders. Termites tunneled through the 91.4 cm length of Tygon tube and reached the feeding site in three days. After reaching the feeding site, termites constructed mud tubes to reach the wood blocks (Figure 1). These tubes were constructed by transporting soil particles from walls of the earlier portions of the tunnel. Termites tunneled significantly longer distances during the first day (36.7 cm ± 3.4) than during the second day (20.9 cm ± 2.4) or the third day (20.9 cm ± 2.2) ($F = 11.15$, $P = 0.0001$, df = 2,44). Termite movement consisted mainly of walking inside the constructed tunnels between the two sites, in addition to local movements in the group and feeding arenas.

Behavioral observations and mortality post-treatment. A significant reduction in termite activity, as indicated by Termatrac readings and visual observations, occurred in the two fipronil treatments compared to the control, where little or no change in activity occurred. Post-treatment, Termatrac reading data were collected from examining the graphs generated from the Termatrac device for each treatment to determine whether there was termite movement. Most termites showing fipronil intoxication (Quarcoo et al. 2010) ultimately

Fig. 1. Termites constructing mud tubes pre-treatment three days after reaching wood blocks in the feeding site cylinder.

ended up dying in the group site rather than dying inside the treated tunnels or feeding sites. Therefore, Termatrac readings from group sites were used to analyze the efficacy results of the experiment.

Visual observations of termite activity in the groups, tubes, and feeding sites were corroborated by Termatrac T3i readings from the group sites (Figure 2). The decrease in termite movement was considerably faster in the feeding sites and inside Tygon tubes compared to the group site. Termites in Tygon tubes and at feeding sites showed little or no behavioral change before a complete absence in these two locations. On the other hand, termites at the group site appeared to aggregate and display the general signs of fipronil intoxication (Hu et al. 2006) within 24 h of treatment in response to the 0.30 mg dose and at 48 h to the 0.15 mg dose, until no movement was reached as indicated by Termatrac readings. The Termatrac T3i is capable of detecting termite movement and not the size of termite populations. The generated readings showed decreasing peaks and wave heights over time, indicating the decrease in termite movement intensity (Figures 2 & 3). Fipronil exposure also resulted in cessation of tunneling activity. Termites exhibiting signs of fipronil intoxication appeared to be less mobile and unable to construct tunnels compared to unaffected termites.

Dose effect. The abnormal behaviors and cessation of activity/movement were observed earlier at the higher dose (0.30 mg/group) than with the lower dose (0.15 mg/group). Tunneling construction deceased in the high dose (0.30 mg) within 24 h after treatment, whereas in the low dose (0.15 mg) tunneling

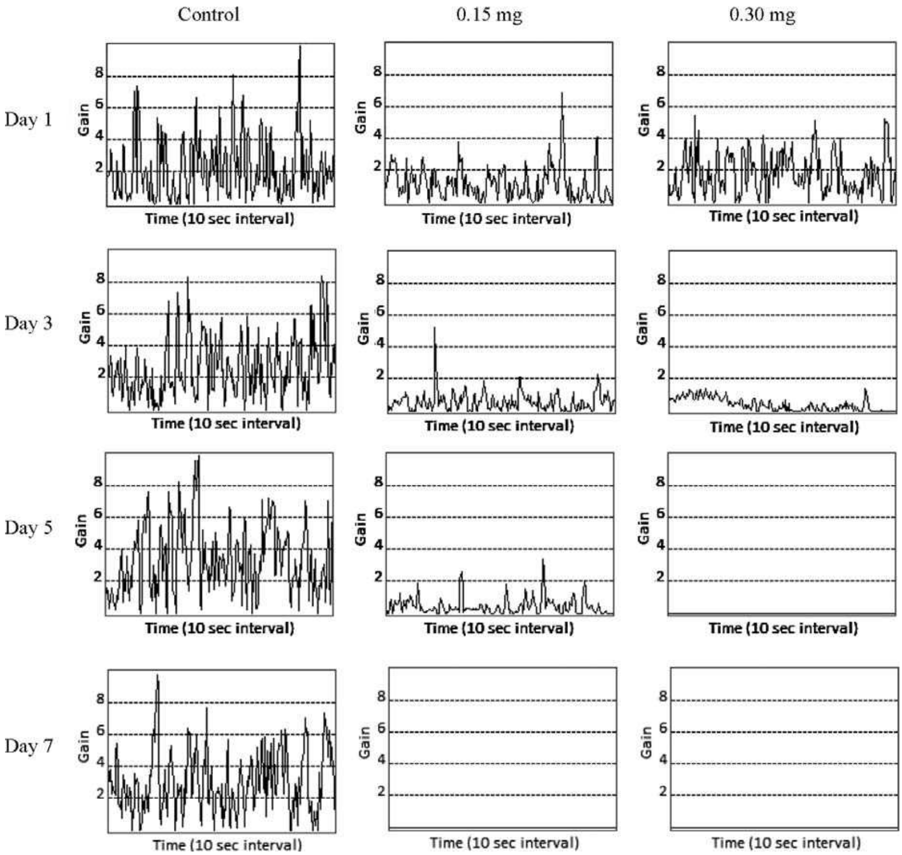

Fig. 2. Generated graphs by Termatrac T3i post-treatment to record eastern subterranean termite movement intensity. The graphs are represented by gain versus time in 10 sec intervals measured in the group site cylinder. Most termites showed fipronil intoxication in the group site rather than inside the treated tunnels or feeding sites.

construction decreased within 48 h after treatment. Visual observations and Termatrac reading showed that the higher dose resulted in no movement at five days post treatment; whereas the lower dose resulted in a longer display of disorientation and no movement at seven days post treatment (F = 141.66, $P <$ 0.001, df = 3). Dismantling of the experimental units revealed only one out of ten units had a few live workers hiding in a feeding gallery inside a wood block, indicating a 90% accuracy of Termatrac readings.

Discussion

This study tested a newly developed dry RTU formulation of fipronil against eastern subterranean termites using a localized treatment technique. It demonstrated for the first time the effect of locally treating a single tunnel with

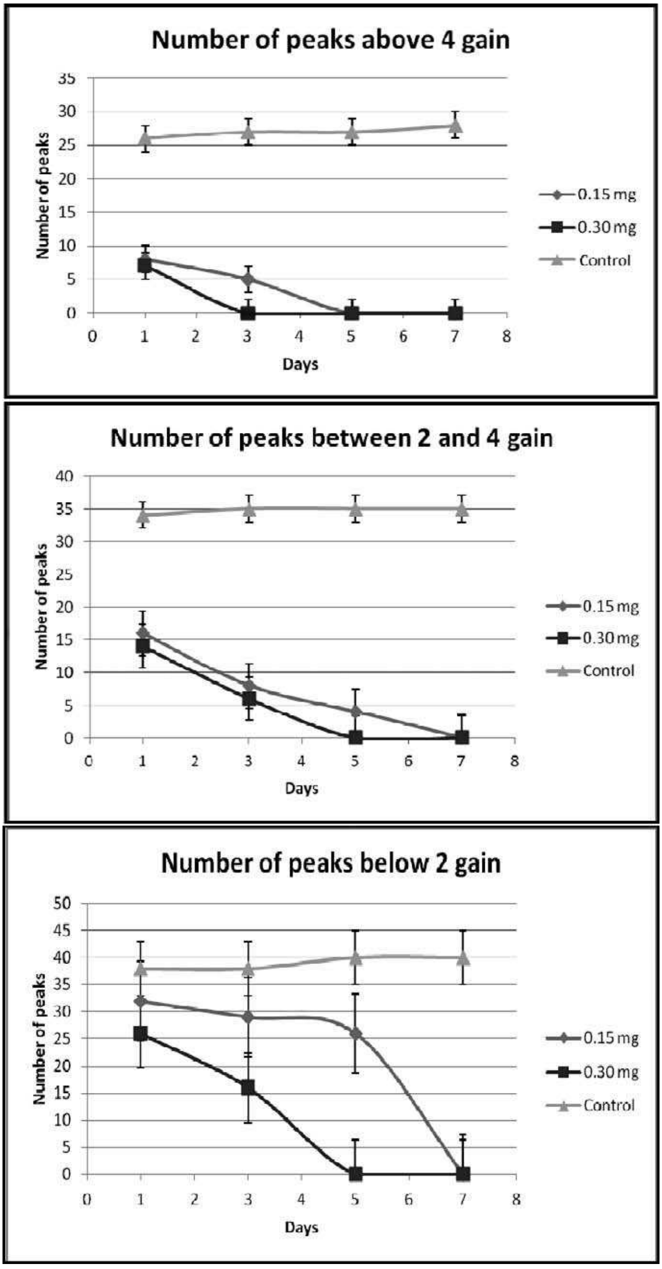

Fig. 3. Number (mean ± SE) of peak readings below 2 gain, between 2 and 4 gain, and above 4 gain from each Termatrac T3i graph of the group site cylinders post-treatment.

dry RTU fipronil formulation against eastern subterranean termites. The results from this study demonstrated that locally treating a single tunnel with dry RTU fipronil near feeding sites was effective for control of termite groups. Currently, liquid fipronil soil treatments are commonly used to control eastern subterranean termite population, which involves trenching around the perimeter of a structure and/or drilling holes at regular intervals into the foundation block and slabs (Rambo 1985). The success of soil treatment largely relies on a uniformly treated soil barrier, which can be affected by soil pH, clay and organic matter content, and soil moisture (Rambo 1985). Eastern subterranean termites are widely dispersed in the soil, making accurate and effective soil treatment difficult to achieve (Myles 1996). It is tactically and economically advantageous to treat above-ground tunnels with dry RTU fipronil formulation. The present study provides evidence that termites at both group sites and feeding sites suffered 100% mortality in 5–7 d post-treatment of a single tube.

Caution should be taken in applying these laboratory findings to termite control in the field. Potential limitations of treating above-ground tunnels with a dry RTU formulation include the blockage of tunnels by dead termites or clotting of dry RTU formulation, which may reduce the residential efficacy and control effectiveness. In addition, this study was conducted with a termite group population of 500 individuals, in contrast to the millions of termites in a typical colony in the field. Furthermore, the experiment was conducted in a laboratory setting in which workers were in confined locations, whereas within the field, termite colonies have multiple feeding sites, broad foraging areas, and multiple above-ground tunnels at various locations.

The time period we used to evaluate the treatments (7 d) was considerably shorter than for some other studies that employed a 30-d interval (Lewis & Rust 2009). Therefore, we hypothesize that all of our treatments would have shown 100% mortality given more time. According to Ibrahim et al. (2003), Song & Hu (2006), and Saran & Rust (2007) lower doses of termiticide require a longer period of time to manifest symptoms of poisoning.

Many previous studies have investigated the influence of horizontal transfer of fipronil between termites. Saran & Rust (2007) reported that the maximum transfer of fipronil from donors to recipients in western subterranean termites, *Reticulitermes hesperus* Banks, occurred within the first 24 h. Quarcoo et al. (2012) reported that higher fipronil concentrations resulted in faster onset of abnormal behaviors, morbundity, and death. Hu (2005) demonstrated the efficacy and non-repellency of fipronil-treated soil and various concentrations against field-collected eastern subterranean termite. Song & Hu (2006) reported that the time required for full expression of transferable lethal effects of fipronil on untreated termites increased as the dose on treated termites decreased at given donor-recipient ratios, and the efficacy of fipronil soil treatment was positively correlated to applied concentration and soil-barrier thickness.

To date, the only study that investigated the effect of a dry RTU fipronil formulation was tested on the drywood termite, *Incisitermes snyderi* (Kalotermitidae), in naturally infested cypress lumber using localized treatment techniques (Hickman & Forschler 2012). They reported that the dry RTU fipronil formulation performed as effectively as foam imidacloprid, and they provided evidence of elimination of infestation without removal of every board that was treated. They proposed that less than 100% elimination was due to the difficulty in treating all termite galleries and the short duration of the trial, which may not have allowed enough time for unexposed termites to contact treated galleries. For subterranean termites, the above-ground tunnels are obvious and can be accurately treated with dry RTU formulation. Our study showed that treatment with a dry RTU formulation of fipronil to a single active tube connected to two locations where termite workers and soldiers aggregate eliminated all termites to a point where no population recovery could occur. Future study will test this treatment concept on termite colonies in laboratory and then in the field.

In conclusion, this study helped us gain a good understanding of the effectiveness of treating mud tunnels using powdered formulations against eastern subterranean termites. The approach of localized treatment is an important step in reducing termiticide exposure to humans and the environment. It is critical for termite control methods to be continuously researched and to reflect newer, more advanced technologies.

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