

## **Tunneling and Activity of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) Exposed to Low Concentrations of Nonrepellent Termiticides**

by

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### **ABSTRACT.**

Tunneling and activity bioassays of chlorfenapyr, fipronil, and imidacloprid treated sand were conducted in the laboratory using *Reticulitermes flavipes* (Kollar). Termites tunneled significantly less through sand treated with 1.0 ppm concentrations of fipronil and imidacloprid compared to the control and chlorfenapyr. Mortality after 7 d was 57, 25, and 29% for fipronil, chlorfenapyr, and imidacloprid, respectively. In a second experiment, termite activity was determined from sound events detected in an acoustical event detection system over a 48 h period after termites were placed on sand treated with 1.0 or 10.0 ppm concentrations. Activity of termites declined to near zero within 3 and 9 h when exposed to 1.0 ppm imidacloprid- and fipronil-treated sand, respectively. Termites in the 1.0 ppm chlorfenapyr treatment showed a decline in activity to near zero after 7 h, followed by an increase in activity and another decline at 12 and 13 h only to increase once again and remain significantly above zero for the remainder of the 48 h period. Mean termite mortality 7 d after termites were placed on treated sand was 100, 99, and 76% for fipronil, chlorfenapyr, and imidacloprid, respectively. Termite activity declined to near zero 2, 8, and 9 h after exposure to 10.0 ppm imidacloprid-, chlorfenapyr-, and fipronil-treated sand, respectively. Mortality associated with the 10.0 ppm concentration, recorded after 4 d, was 100% for fipronil and 92% for chlorfenapyr. Mortality due to imidacloprid exposure was 100% after 7 d.

**Key Words:** nonrepellent termiticide, toxicity, termite activity

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## INTRODUCTION

The use of slow-acting termiticides for the protection of structures against termite attack has received much attention since the development of the newer nonrepellent termiticides. The concept of delayed toxicity initially began with the evaluation of baiting systems for termite control because a slow-acting toxicant was needed to allow foragers contacting the toxicant in the bait to travel back to the colony to transfer it to nestmates before dying (Esenther & Beal 1978, Beal & Esenther 1980). Myles (1996) developed a treat and release method in which a resinous formulation of sulfluramide, a slow-acting stomach poison, was applied to termites captured in monitoring stations. After treatment, the termites were released back into traps to transfer the toxicant to nestmates. He observed 95% mortality in lab tests when 5 or 10% of termites were treated with this coating and >60% suppression of field populations of *R. flavipes* in the first year after treatment.

Su *et al.* (1987) defined slow-acting insecticides as those killing 90% of the treated individuals within 14 d, producing a broad effective lethal-time 90%. Newer termiticides such as chlorfenapyr, fipronil, and imidacloprid have been categorized as slow-acting and attention has been given to the possibility of transfer of these compounds among nestmates with the ultimate result of colony elimination. While laboratory studies (Ibrahim *et al.* 2003, Shelton & Grace 2003, Su 2005, Mulrooney *et al.* 2007) and at least one field study (Potter & Hillery 2001), have provided some evidence of termiticide transfer, the concept of colony elimination has not been demonstrated in the field. Recently Saran and Rust (2007) in an extensive study of toxicity, uptake, and transfer, provided evidence that fipronil could not be transferred between nestmates in sufficient amounts to cause colony elimination.

Nonrepellent compounds are being selected more often as soil treatment for termites than repellents because they do not seem to disrupt termite foraging in the treated soil zone (Kard 2003). While slow-acting nonrepellent termiticides may not eliminate colonies, their imperceptibility to termites and delayed toxicity does have the possible advantage of reducing termite populations more than repellent compounds. Termites rarely contact soil treated with repellent compounds and therefore do not acquire lethal concentrations.

The application of a termiticide to soil rarely results in a uniform distribution of the active ingredient throughout the treated zone and as dissipation and leaching occur, the distribution becomes less uniform. As a result, termites that begin foraging through a treated zone are likely to initially encounter low concentrations of the termiticide. The amount of termite tunneling through treated soil and the associated decline in termite activity should be determined by the concentration of the termiticide in the soil and its speed of action. In this study, we measured the amount of termite tunneling and the decline in termite activity after exposure to low concentrations of three registered nonrepellent termiticides. The distance tunneled by termites and their decreased activity were used to estimate the relative impenetrability of low concentrations of nonrepellent termiticide treatments.

## MATERIALS AND METHODS

### **Termites.**

Sections of pine logs containing termites were collected from both urban and forested areas around Starkville, MS and held at ambient temperature (~24°C) in galvanized trashcans in the laboratory. The termites were identified as *Reticulitermes flavipes* (Kollar) from soldier morphology (Hostettler *et al.* 1995). Two different colonies were used for each bioassay.

### **Termiticides.**

For tunneling bioassays, formulations of chlorfenapyr (Phantom, BASF Corp.), fipronil (Termidor 80WG, BASF Corp.), and imidacloprid (Premise 75, Bayer Environmental Science) were mixed with distilled water at such concentrations to result in 1.0 ppm in silica sand (wt. AI/wt. sand).

### **Tunneling Bioassay.**

For this study, 20 ml of each 1.0 ppm termiticide mixture were applied to 100 g of sand in resealable plastic bags. Experimental units used were similar to those used by Jones (1990). Each arena consisted of a thin layer (2 mm thick) of treated sand sandwiched between a glass and a plexiglass plate (Fig. 1). The inside dimensions of the arena were 5.1 by 0.2 by 11.5 cm. Tygon tubing (7.5-cm by 0.3-cm diameter) connected arenas to nest containers (Fig. 1) composed of Tenite<sup>®</sup> butyrate (U.S. Plastic Corp., Lima,

OH), 3.5-cm diameter by 6.5-cm height, that contained 25 g of untreated sand at 20% moisture. Two hundred termites greater than third instar were added to each nest container. Thin wooden sticks were inserted into the sand in nest containers in a manner to help guide termites to the openings of the tygon tubes and encourage them to enter the tunneling arenas. There were four replicates of each termiticide plus a water-only control. Distances tunneled were measured after 7 d and mortality recorded.

### Activity Bioassay.

In a separate study, 2 ml of each termiticide mixture at both 1.0 and 10.0 ppm concentrations were applied to 10 g of sand in 3.5 cm (diam.) plastic Petri dishes. The sand was allowed to dry overnight at 25°C to allow evaporation of solvents contained in the formulation. The treated sand was then poured into butyrate tubes (3.5 cm diameter by 6.5 cm length) covering a small wooden

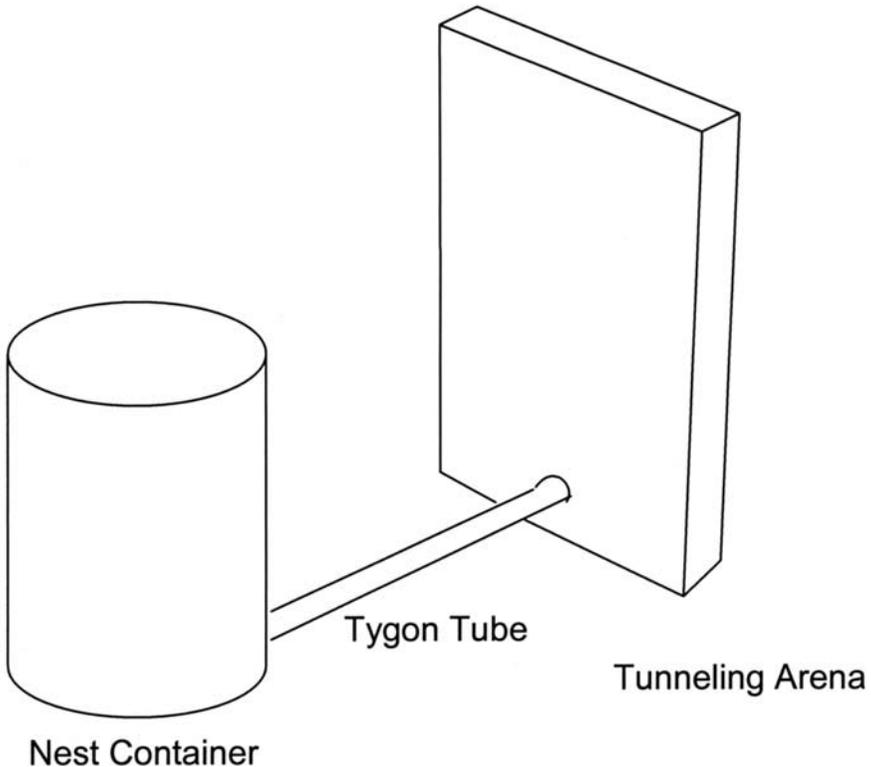


Fig. 1. Nest container and tunneling arena.

block (12 by 12 by 5 mm) placed on the bottom of the tube. The sand was moistened with 2 ml of distilled water. Worker termites greater than third instar (150) were placed in the tubes. The tubes were then capped with plastic caps (3.8-cm i.d. by 0.3-cm inside length) and placed in the Acoustic Detection System (AED-32; described below). The outside surface of the bottom cap was covered with a thin film of stopcock grease (Lubriseal<sup>®</sup>, Thomas Scientific, Swedesboro, NJ) to seal the tube and the microphone before placement in sound-proof boxes. A section of PVC pipe (82.5-cm o.d. by 118.5-cm length) was placed over each microphone and tube isolating sounds from individual tubes. The tubes were held at ambient room temperature. Mean temperatures inside sound-proof boxes ranged from  $20.08 \pm 0.01$  to  $27.11 \pm 0.03^{\circ}\text{C}$  as recorded by HOBO<sup>®</sup>, H08-001-02, dataloggers (Onset Computer Corp., Bourne, MA). While the sound events were only recorded for 2d (see below), the bioassay of 1.0 ppm concentrations were terminated after 7 d. Bioassays of 10.0 ppm concentrations of chlorfenapyr and fipronil were terminated after 4 d. Bioassay of imidacloprid at 10.0 ppm was not stopped until day 7 because it is slower acting than chlorfenapyr and fipronil. There were four replicates of each treatment plus a water-only control.

### **Acoustical Detection System.**

The AED-32 is a 32-channel acoustical event detection system (AED-32) developed by the National Center for Physical Acoustics at the University of Mississippi (Mulrooney *et al.* 2007). It was used to detect and monitor termite activity during the second set of studies. The AED-32 is comprised of 32 front-end commercial electret microphones embedded in a stethoscope head, each with an equivalent sensitivity of approximately 50 mV/Pa. Sixteen microphones (4.0 cm diameter) were mounted on a soft rubber mat (0.5-cm thick) that covered the floor of a sound-proof box (40 by 40 by 18 cm, o.d.), which is housed within a second sound-proof box (60 by 60 by 33 cm, o.d.). The remaining 16 microphones were housed in an identical set of sound-proof boxes. The two sets of boxes rested on soft rubber cushions on a concrete floor of a carpeted room (3 by 4 m). Sounds detected by the microphones first passed through high-gain voltage amplifiers and were then digitized with a 12-bit analog-to-digital (A/D) converter (model DT3002 PCI card, Data

Translation, Marlboro, MA) at a sampling rate of 10 kHz (the analog gain of each A/D input channel was set to unity).

Insect activity was monitored using a custom computer algorithm containing a spectral subtraction routine. Software parameters were set to record events only within the bandwidth of 500 Hz to 2000 Hz. Signal voltages less than 0.5% of the A/D dynamic range of 0 to  $10 V_{\text{rms}}$  (under-range threshold) and greater than 98% of the range (clipping threshold) were not processed. The activity detected by this system included all sounds made by termites as they fed and moved about each butyrate tube containing treated sand and a wooden block. In our tests, the AED-32 acquired the number of acoustical events from the microphones every 10 seconds for 48 h.

### Data Analysis.

The length of tunnels and associated mortality were analyzed using PROC GLM (SAS Institute 2001). Means were separated using least significant difference ( $\alpha = 0.05$ ). Mean sound events (termite activity) averaged over 10 sec sampling periods were analyzed using PROC MIXED (SAS Institute 2001). A *t* test of least square means was used to determine the times when sound events were not significantly different from zero. Mortality data in the activity bioassays were used to confirm decline in activity indicated by acoustical data and were not subjected to ANOVA.

## RESULTS AND DISCUSSION

### Tunneling Bioassay.

The mean distance termites tunneled and the associated mortality are shown in Table 1. Termites tunneled significantly less ( $F = 9.48$ ;  $df = 3, 28$ ;  $P = 0.0002$ ) through sand treated with 1.0 ppm concentrations of fipronil and imidacloprid compared to the control and chlorfenapyr which were not significantly different from each other.

Table 1. Mean ( $\pm$ SEM) distance tunneled and termite mortality in 7 d laboratory bioassays of nonrepellent termiticides.

Treatment (1 ppm)	Tunnels (mm)	Mortality* (%)
Control	193.1 $\pm$ 36.5 a	16.4 $\pm$ 2.0 b
Chlorfenapyr	156.0 $\pm$ 20.1 a	25.2 $\pm$ 6.3 b
Fipronil	51.9 $\pm$ 16.7 b	57.4 $\pm$ 7.8 a
Imidacloprid	53.8 $\pm$ 13.1 b	28.7 $\pm$ 11.0 b

\*Dead + moribund

Means in a column followed by the same letter are not significantly different as determined by LSD ( $P < 0.05$ ).

In a study by Yeoh and Lee (2007), *Coptotermes gestroi* (Wasmann) workers in glass tubes containing a 2.0-cm section of sand treated with 1.0 ppm of either chlorfenapyr, fipronil, or imidacloprid plus an additional 21 cm of untreated sand had penetrations of 100, 88, and 69%, respectively. Also, in a penetration study of fipronil, Hu (2005) observed that both *C. formosanus* Shiraki and *R. flavipes* termites were only able to completely penetrate 50 mm thicknesses of soil when fipronil concentrations were  $\leq 10$  ppm.

Fifty-seven percent of termites in containers connected to tunneling arenas containing 1 ppm fipronil died after 7 d. This was significantly ( $F = 5.26$ ;  $df = 3; 28$ ;  $P = 0.0057$ ) greater than the control (16%), chlorfenapyr (25%), and imidacloprid (29%) which were not significantly different from each other. In Yeoh and Lee (2007) *C. gestroi* mortalities were 100, 48, and 12% for fipronil, chlorfenapyr, and imidacloprid, respectively at 1 ppm. Differences in mortality between Yeoh and Lee (2007) and our study may be due to differences in experimental design as well as the termite species used. Yeoh and Lee (2007) introduced *C. gestroi* termites into a tube containing treated sand; whereas in our study, *R. flavipes* termites had an option of whether or not to leave the nest tube and enter treated sand in the tunneling arena.

In addition, fipronil's ability to be transferred to nestmates and its toxicity at low concentrations may have contributed to increased mortality if termites tunneling through fipronil treated sand in the tunneling arena transferred it to nestmates (Shelton and Grace 2003, Mulrooney *et al.* 2007, Su 2005, Saran and Rust 2007). The toxicity of fipronil to subterranean termites has been documented by several researchers. Ibrahim *et al.* (2003) determined the 72 h  $LD_{50}$  of fipronil to be 1.36 ng/insect in topical bioassays using *C. formosanus*. Osbrink *et al.* (2001) established the  $LT_{50}$  of fipronil to *R. virginicus* to be an average of 271 min when termite workers were placed on filter paper treated with 630.65  $\mu\text{g}/\text{cm}^2$  of fipronil. 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.

Low concentrations of chlorfenapyr and imidacloprid have not been shown to be as toxic to termites as fipronil. For example, Rust and Saran (2006) in bioassays using *R. hesperus* determined 7-d  $LD_{50}$ 's of chlorfenapyr to be 29.98 ng per termite compared to 14.01, 3.21, and 0.16 for chlorpyrifos, cypermethrin, and fipronil, respectively. Seven day mortalities of *R. flavipes*

continuously exposed to 1.0 and 5.0 ppm concentrations of imidacloprid in sand were 7.8 and 22.3%, respectively (Ramakrishnan *et al.* 2000).

### Activity Bioassay.

Mean sound events recorded during 10 sec sampling periods for each of the first 48 hours of the bioassay of 1.0 ppm concentrations of the termiticides are shown in Fig. 2. All of the termiticides had overall mean sound events that were significantly ( $F = 17.76$ ;  $df = 3, 3$ ;  $P = 0.0206$ ) different from the control and the treatment\*time effect was significant ( $F = 1.72$ ;  $df = 141, 1316$ ;  $P < 0.0001$ ). The times at which mean sound events (activity) was not significantly different ( $P < 0.05$ ) from zero are indicated by an asterisk. Activity of termites exposed to imidacloprid declined to near zero within 3 h of exposure (Fig. 2). Termites in the chlorfenapyr treatment showed a decline in activity at 7 h after exposure followed by activity that increased significantly above zero and then again declined at 12 and 13 h only to increase once again and remain significantly above zero for the remainder of the 48

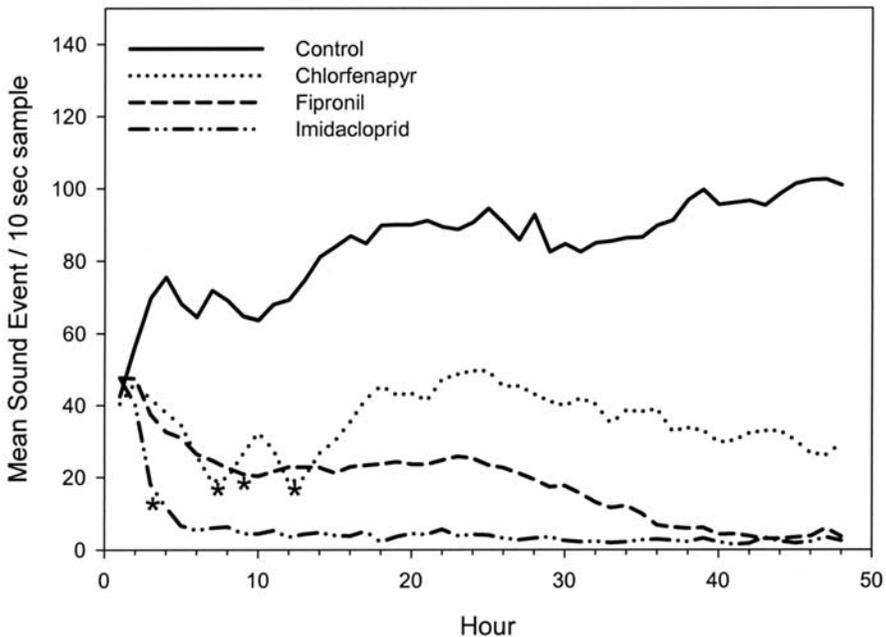


Fig. 2. Termite activity after exposure to 1.0 ppm concentrations of nonrepellent termiticides. \*Time at which activity decreased to a level not significantly different ( $P < 0.05$ ) from 0.

h period (Fig. 2). Decline in termite activity to near zero occurred 9 h after exposure to fipronil (Fig. 2).

Mortality was recorded after 7 d to show that there was no continuation of termite activity after 48 h. Seven days after termites were placed on sand treated with 1.0 ppm mortalities for fipronil, chlorfenapyr, and imidacloprid were  $100 \pm 0.0$ ,  $98.7 \pm 0.7$ , and  $76.5 \pm 5.8\%$ , respectively. Control mortality was  $9.2 \pm 0.7\%$ .

When termites were exposed to 10.0 ppm concentrations of the termiticides, termite activity declined to near zero 2 h after exposure to imidacloprid (Fig. 3). Termite activity in the chlorfenapyr treatment was not cyclic as with the 1.0 ppm concentration; activity declined to levels near zero after 8 h of exposure and remained there for the duration of the 48 h period. Activity after exposure to fipronil declined to near zero after 9 h, the same as that when termites were exposed to a 1.0 ppm concentration.

Mortality, recorded after 4 d, on sand treated with 10.0 ppm concentrations was  $100 \pm 0.0\%$  for fipronil,  $92.4 \pm 3.0\%$  for chlorfenapyr, and  $5.6 \pm 0.9$

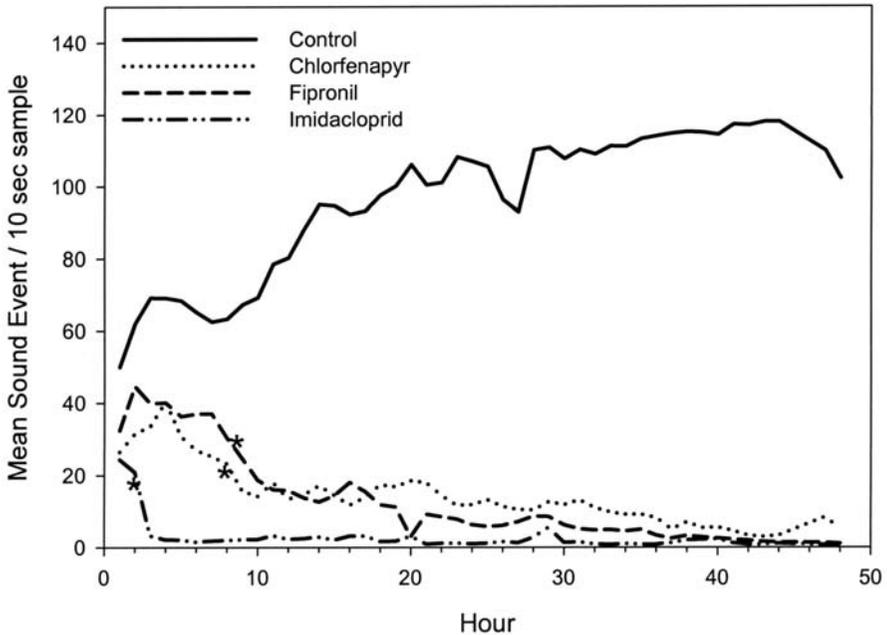


Fig. 3. Termite activity after exposure to 10.0 ppm concentrations of nonrepellent termiticides. \*Time at which activity decreased to a level not significantly different ( $P < 0.05$ ) from 0.

for controls. Because the rate of kill of termites due to imidacloprid toxicity is slower (Ramakrishnan *et al.* 2000) compared to that of chlorfenapyr and fipronil, mortality due to imidacloprid exposure was not recorded until 7 d after exposure. All termites were dead at this time.

The activity of termites placed on sand treated with 10 ppm imidacloprid showed the fastest decline in activity (2 h) among the termiticides tested (Fig. 3). As a result, imidacloprid would not be expected to have as great a transfer potential as fipronil, because termites that are immobilized by imidacloprid would not be able to mingle with unexposed nestmates outside the treated zone and therefore would not be expected to decrease termite populations to the same extent as fipronil. The delayed action of fipronil was also demonstrated when a 24-h exposure of *R. flavipes* to filter paper treated with 1 ppm fipronil resulted in 31% mortality after 24 h and 84% after 7 d (Remmen and Su 2005).

Paralysis of insects is characteristic of imidacloprid toxicity when imidacloprid attaches to nicotinic receptors causing impairment of the nervous system (Schroeder and Flattum 1984). Immobility of termites exposed to imidacloprid was reported by Thorne and Breisch (2001) when 95% of termites exposed for 4 h to 10- and 100-ppm concentrations of imidacloprid failed to tunnel after being placed on untreated sand during a recovery period. Imidacloprid limited termite penetration of 50-mm thicknesses of soil treated with 1.0 and 10.0-ppm concentrations to 20 and 0%, respectively, with resultant mortalities of 58 and 100% (Gahlhoff and Koehler 2001).

As termites forage into a treated zone in the soil, lower concentrations of a termiticide are encountered at the outer edges of the treated zone due to leaching and dissipation after application. Based on this study, termites encountering concentrations of imidacloprid, the nonrepellent shown to have the fastest reduction in termite activity, as low as 1.0 ppm would cease activity after 3 h and tunneling would be reduced to an average of 5.4 cm. If these termites were not able to retreat from the treated zone, 76% of them would be expected to die within 7 d. Contrasted with fipronil results at 1.0 ppm, termite activity would cease after 9 h, with a distance of 5.2 cm tunneled through the soil. All of the termites in the treated zone would die after 7 d if they were unable to leave. Activity of termites exposed to chlorfenapyr was reduced to near zero only with the 10 ppm concentration; at this level, all

termite activity declined to near zero after 8 h and killed 92% of the termites after 4 d.

While the termiticides tested in this study differ somewhat in the rate of onset and degree of toxicity, their efficacy in the USDA Forest Service termiticide tests has been well established (Wagner *et al.* 2008). Nonrepellent termiticides will continue to be popular choices for termite control because they are undetectable to termites and are low in toxicity to humans. Current labels of Termidor (fipronil) and Premise (imidacloprid) allow for exterior perimeter/limited interior application of these termiticides around structures, a departure from requirements of repellent termiticides which call for pre-construction as well as perimeter treatments. The popularity of these termiticides with Pest Management Professionals is certain to increase due to the conveniences of exterior perimeter/limited interior application.

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