

Activity of *Reticulitermes flavipes* (Isoptera: Rhinotermitidae) Exposed to Nestmates Treated with Slow-Acting Nonrepellent Termiticides

by

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ABSTRACT

A 32-channel acoustical event detector was used to monitor termite activity in donor/recipient bioassays of slow-acting nonrepellent termiticides. Acoustical detection of termite activity illustrated termite mortality over time, attributed to termiticides moving from donors to recipients. In the first of two donor-recipient experiments, worker termites (donors) were exposed for 1.0 h to sand treated with 50 ppm concentrations of fipronil or imidacloprid. Donors were then combined with recipients at 5% of the total population of 200 workers. Termite activity was monitored for 14 d. Only fipronil significantly reduced termite activity (sound events), survival, and wood consumption below controls. In a second donor-recipient experiment, fipronil significantly reduced activity, survival, and consumption below controls when donors (12.5% of the population) were exposed to 1.0 ppm for 1.0 h.

Keywords: termite activity, nonrepellent, termiticide, acoustical event, *Reticulitermes flavipes*.

INTRODUCTION

Insecticides have continued to evolve since the first organophosphates and chlorinated hydrocarbons were developed in the 1940's. This progression has been spurred by the development of insect resistance; consumer demand for safer, more environmentally-benign pest control products; and competition within the chemical industry to discover more efficacious insecticides.

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Newer termiticide chemistries have reduced risks to human health and the environment. Included among these newer products are neonicotinoid, pyrrole, and pyrazole compounds that have been shown to be nonrepellent to termites at labeled rates, unlike pyrethroid termiticides. In addition to being non-repellent, these newer chemistries are slower acting against termites than pyrethroids and organophosphates. It is this combination of nonrepellency and delayed-action that has changed the way these termiticides are thought to affect termites (Potter & Hillery 2001, Wagner 2003).

Esenther & Beal (1974, 1978) investigated a slow-acting toxicant for the elimination of colonies of subterranean termites. Bait blocks impregnated with dechlorane (mirex) and buried in the soil suppressed field activities of *Reticulitermes* colonies. Since then, several slow-acting toxicants including hydramethylnon (Su *et al.* 1982), avermectin B₁ (Su *et al.* 1987), sulfluramid (Su & Scheffrahn 1991), fenoxycarb (Jones 1989), and hexaflumuron (Su 1994) have been evaluated for use in termite baits. Su *et al.* (1987) used the effective lethal time 90% (ELT₉₀), the amount of time required for an insecticide to kill 90% of treated individuals, to characterize slow-acting insecticides for control of the Formosan subterranean termite, *Coptotermes formosanus* Shiraki. They found that slow-acting toxicants exhibited ELT₉₀ values that spanned a broad range of time (1.5–14 d), while acute toxicants had a narrow range of ELT₉₀ values (0.2–2 d).

The nonrepellency of new chemistries has been established in several recent studies. Gahlhoff & Koehler (2001) demonstrated that termites penetrated sand treated with 10 and 100 ppm imidacloprid to 25 and 12.5 mm, respectively. Ibrahim *et al.* (2003) found fipronil, a pyrazole, to be repellent at 0.125%. Their data suggest that fipronil would not be conducive for transfer among workers because of repellency. However, Remmen and Su (2005) observed that fipronil concentrations as high as 64 ppm did not repel *Reticulitermes flavipes* (Kollar) or *C. formosanus* termites and that mortality at 1 ppm was high (89%) indicating that lack of penetration into the treated sand was due to mortality, not repellence. They concluded that ≥ 1 ppm fipronil may provide an adequate barrier for both *R. flavipes* and *C. formosanus*.

Hu (2005) found 100% mortality of Eastern subterranean and Formosan termites within 3 d of treatment with fipronil concentrations of 50 and 100 ppm and after 28 d at 1 ppm. Her results also showed that penetration into

treated sand decreased with increasing concentration. Penetration failure was due to rapid mortality, rather than repellency of fipronil.

Slow-acting soil-applied termiticides could be transferred horizontally if contaminated workers live long enough to carry the compound back to the colony where they may be transferred to nestmates. Thorne and Breisch (2001) observed transfer of imidacloprid when 40% of naïve termite workers were intoxicated after exposure to nestmates exposed to 100 ppm residues of imidacloprid. Shelton and Grace (2003) showed that donor *C. formosanus* workers exposed to sand treated with either imidacloprid or fipronil at 100 ppm significantly increased the mortality of recipients above that of controls.

Su (2005) determined the horizontal transfer of noviflumuron, fipronil and thiamethoxam among laboratory colonies of *C. formosanus*. The lethal effects of fipronil and thiamethoxam were limited to <5 m, while noviflumuron was 100% effective over a 50-m span of the arenas. He concluded that nonrepellent termiticides are less likely than termite baits to significantly suppress colony populations. Similarly, Shelton *et al.* (2006) found that chlofenapyr, while capable of being transferred among some *R. flavipes* colonies, did so only at relatively high concentrations (250 – 500 ppm).

Except for Su (2005), who used digital images of test arenas to determine termite mortality, all the other research on termite efficacy cited in this introduction relied on destructive sampling of experimental units to quantify mortality. Once an experimental chamber is dismantled changes in termite toxicity can not be measured.

This research explored the use of acoustic technology to examine the effects of nonrepellent delayed-action termiticides on termite activity. The system used in this study had been used previously to detect and monitor larval activity in cotton bolls and ant sounds (Hickling & Brown 2000, Hickling *et al.* 2000). This technology offers the potential of continuous monitoring of termite activity during bioassays.

Three experiments were conducted. In the first, the activity of different numbers of termites was monitored. The second experiment was a donor/recipient study in which donor termites were exposed to 50 ppm concentrations of fipronil and imidacloprid. In the third experiment, different numbers of donors were exposed to 1.0 ppm of fipronil. In the second and third experi-

ments, survival and food consumption after exposure to termiticides were also determined.

MATERIALS AND METHODS

Acoustical System

A 32-channel acoustical event detection system (AED-32) developed by the National Center for Physical Acoustics at the University of Mississippi was used to detect and monitor termite activity. 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_{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 insects as they fed and moved about each chamber. In our tests, the AED-32 acquired the number of acoustical events from the microphones every 10 seconds for 14 to 30-d periods.

Ambient Noise

In order to determine the background noise levels prior to data collection, acoustic background measurements were performed both inside the room (with the data acquisition computer's fan noise), and inside each of the sound-proof boxes. A Bruel and Kjaer 2.5-cm condenser microphone,

type 4179 (sensitivity 1.11 V/Pa at 20 dB gain) and a Bruel and Kjaer microphone preamplifier, type 2660 (Anonymous 1985) were fixed at a height of 87 cm above the carpeted floor of the experimental room, and inside each box separately. Frequency spectra were measured using a Bruel and Kjaer type 2032 signal analyzer (Anonymous 1983) (bandwidth 3200 Hz, resolution $\Delta f = 4$ Hz, Hanning window, anti-aliasing filter 25.6 kHz). The acquired spectra were scaled as RMS with no A-weighting applied using 50 averages. A Bruel and Kjaer sound level calibrator type 4231 was used to calibrate the acoustic measurement system to a sound level of 94 ± 0.2 dB at 1000 Hz (all Bruel and Kjaer equipment used in this study were calibrated in accordance with NIST-traceable standards in Norcross, Georgia).

Data were collected using a custom GUI developed in LabVIEW™ 6.0 (National Instruments, Austin, TX) and a National Instruments IEEE-488.2 GPIB card on a laptop computer running Windows® 2000. The background spectra were averaged by:

$$L_{BKGND} = 10 \log_{10} \left[\frac{1}{n} \sum_i^N 10^{\left(\frac{L_i}{10}\right)} \right]$$

Where L_{BKGND} is the averaged sound pressure level of n background measurements, L_i is the sound pressure level measured at point i in the bandwidth, and N is the total number of measurement points (Kinsler *et al.* 2000). The averaged background file was then subtracted from each of the data files collected according to the relation:

$$L_{SOURCE} = 10 \log_{10} \left[\sum_i^n 10^{\left(\frac{L_{DATA}}{10}\right)} - 10^{\left(\frac{L_{BKGND}}{10}\right)} \right]$$

Where L_{SOURCE} is the sound pressure level due to the source(s) only over the measurement bandwidth. Each data file was then converted from logarithmic-to-linear acoustic pressure using:

$$P_{rms} = (20.0 \times 10^{-6}) \times (10^{\left(\frac{L_{SOURCE}}{20}\right)}) \quad [Pa]$$

Where 20 μ Pa is the standard reference threshold value for human hearing (Kinsler *et al.* 2000).

Plots of the pressure versus frequency for the experiment room and each box are shown in Fig. 1. A peak-finding routine was used to locate the peaks

for each frequency component in the spectra. It was observed that the acoustic energy present in the measurement room was dominant in the infrasound region (≤ 20 Hz), indicating that a seismic source internal and/or external to the experimental room was responsible for the observed spectra. The pressure curve for the boxes was essentially the same profile as the room measurement, but the pressures were a bit higher, which likely means the boxes were resonating at the infrasonic mode, possibly from a central air conditioning unit. Only low-level acoustic energy was found within the measurement bandwidth region of interest set in the 32-channel signal processing algorithm (500 – 2000 Hz), indicating very low levels of ambient noise.

Termite Activity

R. flavipes were collected from fallen logs on the Noxubee National Wildlife Refuge near Starkville, MS. The logs were cut into sections (ca 75 cm length) and held in 37 L galvanized cans in the laboratory. Identifications were made using soldier keys (Scheffrahn & Su 1994).

Experimental chambers were Tenite[®] butyrate tubes (U.S. Plastic Corp., Lima, OH), 3.8-cm diameter by 6.4-cm length, containing a small yellow pine-wood disk (28.8 ± 0.04 mm diameter and 5.7 ± 0.2 mm thickness) covered with 25 g of sand moistened to 20%. The top and bottoms of the tubes were tightly capped with plastic caps (3.8-cm i.d. by 0.3-cm inside length). The top cap was perforated to provide air exchange during the test. The outside surface of the bottom cap was covered with a thin film of stopcock grease (Lubriseal[®], Thomas Scientific, Swedesboro, NJ) to provide good contact between 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 ± 0.01 to $27.11 \pm 0.03^\circ\text{C}$ as recorded by HOBO[®], H08-001-02, dataloggers (Onset Computer Corp., Bourne, MA).

An initial test was conducted to determine the sensitivity of the AED-32 to detect termite sounds under our experimental conditions. In this test, 50, 75, 100, or 200 worker termites were placed in tubes along with one soldier. Termite activity was recorded for 30 d. There were five replicates of each grouping of termites.

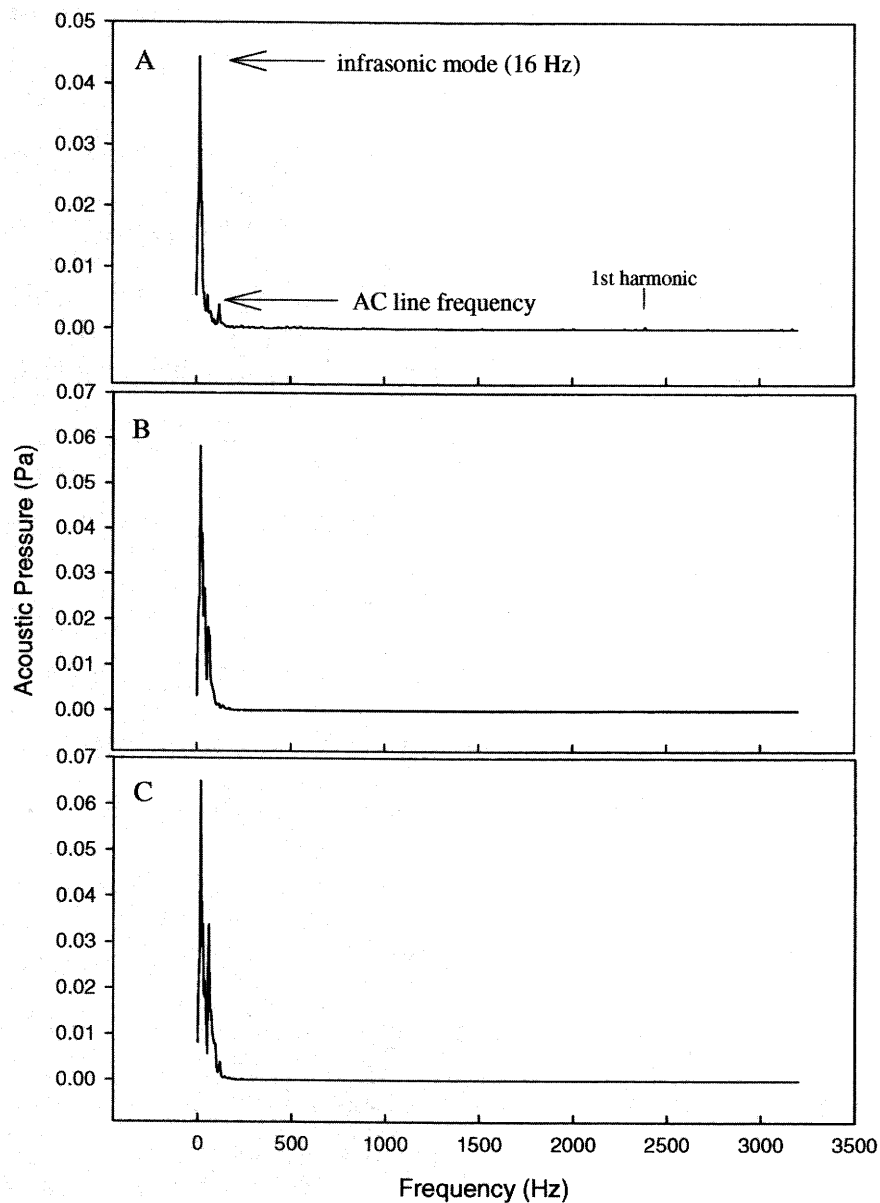


Fig. 1. Acoustic pressure versus frequency for the experiment room (A) and sound proof boxes (B & C).

Donor/Recipient Experiment 1

Dry silica sand (40-100 mesh, Fisher Scientific, Pittsburg, PA) (15 g) was placed in 60-mm diameter plastic Petri dishes. The sand in individual Petri dishes was treated with fipronil (Termidor 80 WG), or imidacloprid (Premise 75) at 50 ppm (wt. AI: wt. sand), then dried in an incubator at 32°C overnight to allow solvents in the formulations to evaporate. It was rewetted to 20% moisture before treatment of termites.

Termite workers (donors) were placed on treated sand in the plastic Petri dishes for 1.0 h. After 1.0 h, termites were gently transferred by aspirator to Petri dishes containing filter paper for a 30-min cleaning period during which termites had an opportunity to dislodge any sand particles adhering to them. After the cleaning period, treated termites (donors) were placed in tubes containing untreated nestmates (recipients) plus one soldier. Two-hundred worker termites plus one soldier were placed in each experimental chamber. The ratio of treated to untreated workers was 10:190 (5%). Controls consisted of untreated workers. Tubes containing wooden disks in moistened sand without termites were used as controls for extraneous noise and as controls for wood consumption estimates. Mean sound events per iteration per day in the control tubes during bioassays was 0.00208 ± 0.00039 .

For each concentration, there were six replicates for each treatment. Termite activity was continuously monitored for 14 d.

At end of each bioassay, termite mortality and food consumption were determined and termite activity summarized. To determine food consumption during the bioassay, wooden disks were oven-dried for 24 h at 100°C and then weighed before placing them in tubes containing termites. After the test period, wooden disks were again oven dried and reweighed. The average decrease in weight of check disks during the test period was 8.1 ± 1.2 mg.

Donor/Recipient Experiment 2

Methods and materials were the same as in experiment 1. Dry silica sand was treated with fipronil at 1.0 ppm, imidacloprid was not included. The ratios of treated to untreated workers were 0:80 (0%), 5:75 (6%), 10:70 (12%), and 15:65 (19%). Termite activity was continuously monitored for 20 d. There were six replicates of each donor:recipient ratio.

Data Analysis

Termite activity was defined as the number of sound events in the frequency range of 500-2000 Hz made by termites during bioassays. For each treatment, average sound events per 10 sec of the signal processing program per day were analyzed to compare the effects of the termiticides and an untreated control over time. These data were analyzed as a randomized complete block with repeated measures using the MIXED procedure in SAS (SAS Institute, 2001) employing a first order autoregressive correlation structure (Littell *et al.* 2006). Least-squares means were used to separate means in the event of significant chemical effects. Termite survival and wood consumption at the end of the test were analyzed using PROC GLM. Least-squares means were separated using the PDIFF option.

RESULTS AND DISCUSSION

Termite Activity

Activity of workers in the initial test increased over time (Fig. 2). Sound events immediately after termites were placed into 3.8-cm tubes were most likely due to workers moving sand and tunneling down to the wooden disk at the bottom of the tube. Termites tended to tunnel between the interior tube wall and the sand. Vibrations resulting from contact with the wall of the tube were transferred through the tube wall to the sensor at the bottom of the tube rather than passing through the sand to the sensor, thus facilitating sound reception. Sound events tended to decrease during the first 3 d of the test and then began increasing as workers most likely began feeding on the wooden disk on the bottom of the tube. Activity by 50 and 75 termites per chamber tended to remain steady while that of 100 and 200 termites increased over time. The greater increase in activity shown by 100 and 200 termites over time could be due to increases in the surface area of the wooden disk as termites create irregularities on the surface. This increase in surface area allows more termites to feed on the wooden disk.

Mean sound events per day among different numbers of termites in a tube over the test period was significantly different ($F = 8.01$; $df = 3, 17$; $P = 0.0014$). The AED-32 did not distinguish differences in sound events between 50 and 75 termites per tube. Mean sound events per day of 200

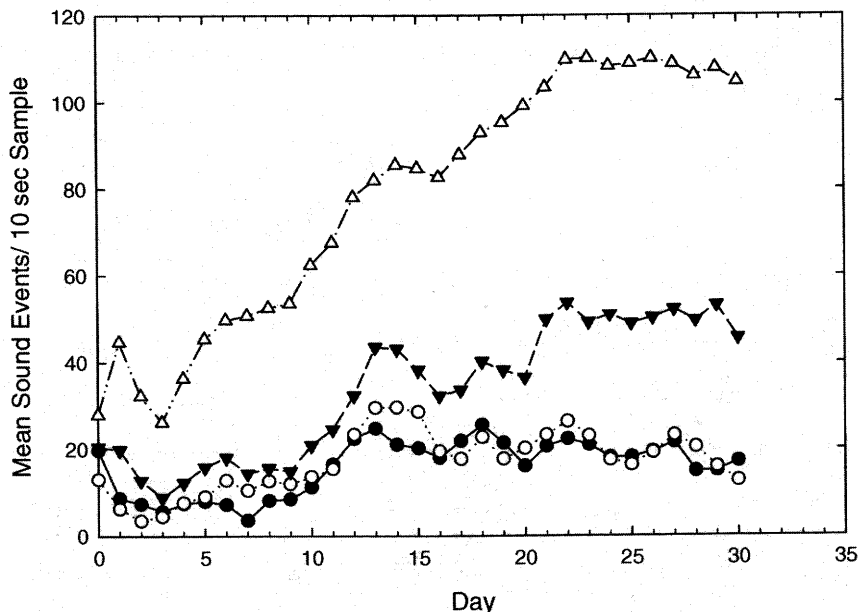


Fig. 2. Activity (mean sound events) of 50 (closed circles), 75 (open circles), 100 (closed triangles), and 200 (open triangles) termite workers as detected by acoustical event detector.

termites was greater than that of 100 termites, and both were greater than that of 50 and 75 termites.

Donor/Recipient Experiment 1

Termite activity (mean sound events/10 sec sample/test period) was statistically different ($F = 9.15$; $df = 2, 16$; $P = 0.0022$) among treatments. Activity of termites exposed to nestmates placed on sand treated with fipronil was less than that of controls or termites exposed to nestmates placed on sand treated with imidacloprid. Termite activity in both controls and imidacloprid treatment decreased initially until day 5 when activity began to increase. Activity after exposure to imidacloprid was slightly less (numerically) than controls but not statistically significant. This perhaps indicates some slight effect of imidacloprid exposure on activity. Thorne and Breisch (2001) reported 82% survivorship of termites (exposed + naïve) exposed for 4 h to 10- and 100-ppm concentrations of imidacloprid in sand. All termites alive after 1 wk appeared to have fully recovered. They also noted that 95% of termites stayed on the sand surface in the recovery container throughout the recovery period. This

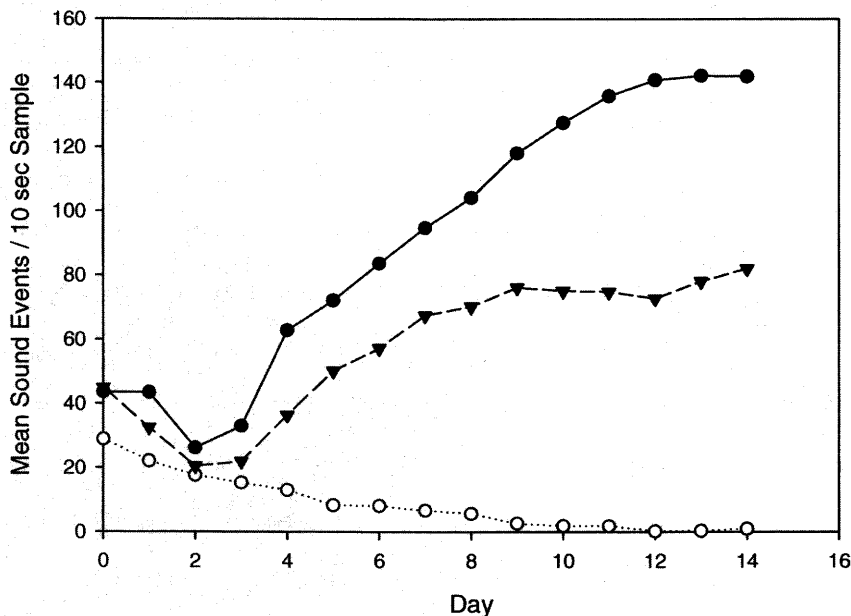


Fig. 3. Activity (mean sound events) of untreated (control) termites (closed circles) and termites after exposure to nestmates (10 donors) placed for 1.0 h on sand treated (@50 ppm) with fipronil (open circles) and imidacloprid (closed triangles).

characteristic of imidacloprid toxicity may explain the slight decrease in initial activity observed in our bioassay.

Termites exposed to nestmates placed on fipronil treated sand showed a decrease in activity from day 0. By day 12, activity was very slight, less than 1 sound event/10 sec. sample/day (Fig. 3).

Survival ($F = 27.67$; $df = 2, 10$; $P < 0.0001$) and wood consumption ($F = 28.85$; $df = 2, 10$; $P < 0.0001$) were significantly different among treatments. Termite survival after exposure to termites placed on fipronil treated sand ($5.8 \pm 5.7\%$) was significantly lower than controls ($94.3 \pm 0.8\%$) and those exposed to nestmates placed on imidacloprid treated sand ($76.4 \pm 15.2\%$). Likewise, termites exposed to nestmates placed on fipronil treated sand consumed less wood (20.6 ± 5.1 mg) than controls (199.8 ± 9.2 mg) or termites exposed to nestmates placed on imidacloprid treated sand (160.8 ± 29.4 mg).

Donor/Recipient Experiment 2

Fipronil had significant effects on termite activity, survival, and wood consumption when 12% and 19% of nestmates were exposed for 1.0 h to sand treated with 1.0 ppm. Termite activity ($F = 4.58$; $df = 3, 9$; $P = 0.0328$), survival ($F = 86.57$; $df = 3, 9$; $P < 0.0001$, and wood consumption ($F = 19.23$; $df = 3, 9$; $P = 0.0003$) were significantly different among donor levels.

Mean sound events per day was greater ($P < 0.05$) for 0% (33.6 ± 7.0) and 6% (17.3 ± 7.0) donors than for 12% (1.7 ± 7.0) and 19% (2.8 ± 7.0) donors. Mean sound events in the 6% donor and 12% donor levels remained below the control throughout the entire bioassay, although significant differences between 0% and 6% levels were not detected (Fig. 4). Mean sound events after exposure to 19% donors dropped below the control at day 4, below the 6% donor level at day 7, and below the 12% donor level at day 11.

The mean number of survivors out of 80 termites was significantly greater ($P < 0.05$) for the 0% donor level (68.8 ± 3.4) than the 6% (17.8 ± 3.4). The 12% (0.8 ± 3.4) and 19% (1.2 ± 3.4) donor levels had significantly lower

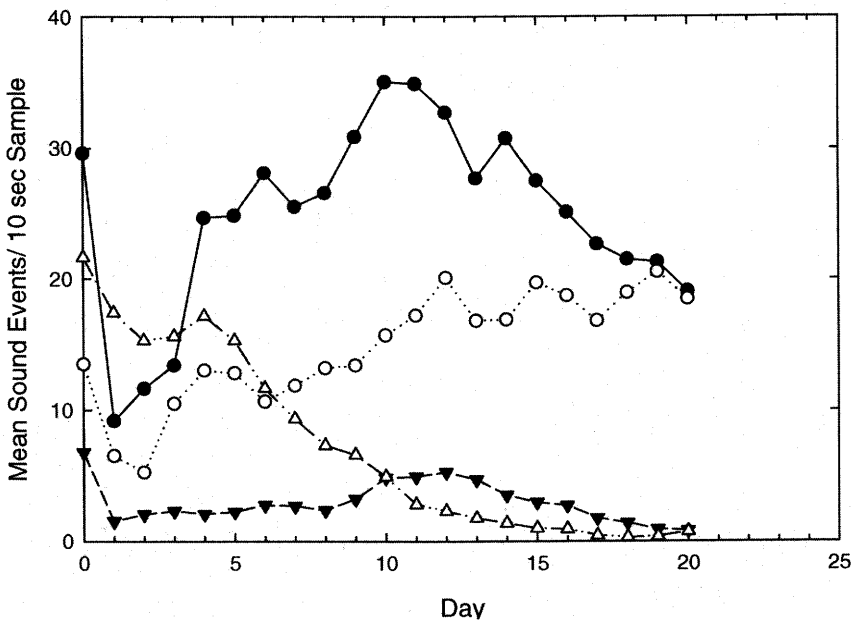


Fig. 4. Activity (mean sound events) of termites after exposure to nestmates (0 donors, solid circles; 5 donors, open circles; 10 donors, solid triangles; 15 donors, open triangles) placed on sand treated with fipronil (1.0 ppm) for 1.0 h.

survivals than the 0%, but were not significantly different from the 6% donor level. Significant differences ($P < 0.05$) in mean wood consumption (mg) among treatments followed the order: 0% donor (141.0 ± 9.7) > 6% donor (98.5 ± 9.7) > 12% donor (57.7 ± 9.7) = 19% donor (47.6 ± 9.7).

Shelton & Grace (2003) conducted donor-recipient bioassays of fipronil and imidacloprid at 1, 10, and 100 ppm using *C. formosanus*, in which donors were exposed to treated sand for 1.0 h. Mortality of recipients after 14 d was different from controls only when donors (5% of recipients) were exposed to 100 ppm concentrations of either fipronil (39%) or imidacloprid (62%). Mortality of *R. flavipes* in the 50-ppm imidacloprid treatment (23.6 ± 15.3 %) in our study was less than that observed by Shelton & Grace (2003), while in the 50 ppm fipronil treatment mortality ($94.2 \pm 5.7\%$) was greater.

Ibrahim *et al.* (2003), in tests of horizontal transmission of fipronil applied at 2.5 ngper termite, showed that the minimum percentage of treated workers (donors) to obtain the maximum horizontal transmission of fipronil among workers was 40%. Near maximum horizontal transmission (99% mortality) occurred after 20 d in the second donor-recipient experiment when a 12.5% donor level was used. However, the amount of fipronil transferred from treated sand to donors after 1.0 h exposure to a 1.0 ppm concentration was unknown.

Su *et al.* (1982) classified termiticides according to the behavioral response they elicited from a colony of termites. Type I (pyrethroids) and Type II (organophosphates) termiticides elicited avoidance behavior, Type I due to repellency of the termiticide and Type II due to dead and decaying termites accumulating in treated areas. Both Types I and II were thought to be most effective as preventative treatments. Type III (Amdro[®]), a slow-acting stomach poison, was thought best used for remedial control because termites would continue to enter treated areas. If a termiticide is to be transferred throughout a colony, it must be a slow-acting, Type III termiticide.

Su (2005) in laboratory bioassays with noviflumuron, fipronil, and thiamethoxam showed that the horizontal transfer of fipronil and thiamethoxam, two nonrepellent termiticides, among workers in a test arena that spanned a 50 m distance was limited to <5 m. Almost all termites in the arena at 0 m from the treatment chamber were dead after 1 wk, and termites stopped entering treated sand, resulting in no additional mortality occurring in arenas

>5 m from treatment, thus indicating these termiticides, at the rates applied (80 and 135 ppm, respectively), were not likely to cause significant colony suppression. Our results showed that when 5% of a population was exposed to sand treated with 25 ppm for 1.0 h, all termite activity stopped within 13 d, which is 6 d longer than that observed by Su (2005). Slower intoxication due to exposure to lower concentrations would allow for greater transfer distances and decrease the accumulation of dead termites in tunnels resulting in decreased avoidance behavior by nestmates.

The amount of termiticide transferred through social interactions as termites tunnel through treated soil is unknown. One thing seems certain; significant effects on colony populations will depend upon a steady influx of toxicant. The abandonment of tunnels in treated areas containing dead and decaying individuals or lining tunnels through treated areas with soil free of termiticide and thus separating the colony from toxic residues could disrupt the flow of the toxicant and negate any prospect of significant horizontal transfer of termiticide among nestmates. While this research has demonstrated that fipronil was transferred from exposed workers to nestmates in amounts that reduced activity, survival, and food consumption, the exact manner in which the social dynamics of termites were disrupted in this study is not known. A considerable number of additional laboratory and field studies on the effects of slow-acting nonrepellent termiticides on termite biology and behavior are needed to fully understand horizontal transfer of soil-applied termiticides.

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