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Influence of Temperature on Rate of Uptake and Subsequent Horizontal Transfer of [^{14}C]Fipronil by Eastern Subterranean Termites (Isoptera: Rhinotermitidae)

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ABSTRACT The effect of temperature on [^{14}C]fipronil uptake and transfer from donor (D) to recipient (R) *Reticulitermes flavipes* (Kollar) (Isoptera: Rhinotermitidae) workers was evaluated. Test chambers used in the fipronil uptake study were constructed from petri dishes containing autoclaved soil treated with 1 ppm [^{14}C]fipronil (1.14 μCi of total radioactivity per petri dish), distilled water, and *R. flavipes* workers. Test chambers were held in environmental growth chambers preset at 12, 17, 22, 27, and 32°C. For the fipronil transfer study, donor termites stained with Nile blue-A were exposed to soil treated with 1 ppm [^{14}C]fipronil for 2 h. Donors were then combined with unexposed recipient termite workers at either 1D:5R, 1D:10R, or 1D:20R ratios. Test chambers consisted of a nest and feeding chamber connected by a piece of polyethylene tube and held in growth chambers at 12, 17, 22, 27, and 32°C. Worker termites were sampled over time and the amount of [^{14}C]fipronil present was measured by scintillation counting. Some degree of uptake and transfer occurred at all temperatures and ratios in this study. The highest level of uptake occurred by termites held at 22–32°C, followed decreasingly by 17 and 12°C. Maximum transfer of [^{14}C]fipronil occurred at the higher ratios ($1{:}5 > 1{:}10 > 1{:}20$) of donors to recipients. Data presented in this study suggest that temperature is one of the key factors affecting the rate of uptake and subsequent horizontal transfer of [^{14}C]fipronil in subterranean termites.

KEY WORDS termiticide, *Reticulitermes flavipes*, horizontal transfer

Subterranean termites cause 80% of all termite damage in the United States, and they are considered to be one of the most economically important structural pests (Su 1991). An estimated $1.6 billion in termite service revenue was reported in 2003 from prevention and control in the United States (Curl, personal communication). The advent of baits and nonrepellent termiticides in the mid-to-late 1990s has dramatically changed the complexity of subterranean termite control techniques. Subsoil applications of liquid termiticides (e.g., organophosphates, carbamates, and pyrethroids) as full barrier treatments are being replaced with noninvasive bait products and spot treatments with slow acting nonrepellent (SANR) termiticides.

Nonrepellent termiticides are commonly used for current prevention and control strategies. Some SANR termiticides have been exclusively used as an exterior perimeter only plus targeted interior treatment. Fipronil (Termidor) has eliminated subterranean termites when used as a targeted treatment in field trials (Kamble and Davis 2005). Fipronil is a phenyl pyrazole insecticide whose mode of action involves disrupting the chloride ion flow through the γ-aminobutyric acid (GABA)-regulated chloride channel (Rhône-Poulenc 1996). Visible symptoms of intoxication of termites to fipronil include twitching and uncontrolled movement, followed by death. Horizontal transfer is one of the reasons used to explain why fipronil has been successfully used as an exterior perimeter application (Potter and Hillery 2002) or perimeter plus targeted interior treatment (Kamble and Davis 2005). Several researchers have reported horizontal transfer of fipronil from exposed termite workers to unexposed nestmates though grooming or contact (Ibrahim et al. 2003, Shelton and Grace 2003, Su 2005, Saran and Rust 2007). The transfer of fipronil is primarily through body contact and transfer by trophallaxis is not significant (Saran and Rust 2007).

Lethal transfer of fipronil between cohorts occurs $\leq$5 m from a treated zone (Su 2005). In addition, fipronil concentrations must be $>$10 ppm to observe mortality in recipient termites (Shelton and Grace 2003). However, at higher fipronil concentrations the onset of a symptomatic response may be observed rather quickly and can prevent toxicant transmission through the entire colony. Fifty percent mortality in termite populations occurs within 24 h when treated topically with 0.7 ng of fipronil (Saran and Rust 2007).

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The horizontal transfer of a toxicant is associated with a complex relationship of factors including the insecticide toxicity, dose, time of exposure, and on onset of toxic symptoms in addition to other natural factors found under field conditions (Rust and Saran 2006).

In an earlier study, we investigated the influence of temperature on transfer of noviflumuron (Spomer and Kamble 2006). This is the first study designed to address the influence of temperature on fipronil uptake and transfer in termite colonies. We used radiolabeled [^{14}C]fipronil to investigate horizontal transfer of fipronil in *R. flavipes* laboratory groups. The objectives of this study were to determine the influence of temperature on the rate of uptake and subsequent transfer of fipronil from donor to recipients.

Materials and Methods

Termite Collection and Rearing. Termite infested logs were collected from Wilderness Park Recreation Area in Lincoln, NE, and transported to the laboratory. Termites were extracted from the logs and maintained in Plexiglas containers (35 by 25 by 10 cm) provisioned with moistened sand and corrugated cardboard and held in complete darkness at 24°C. Termites were allowed to acclimate for ≈48 h before initiating experiments. The termites were confirmed to be *R. flavipes* by using soldier morphology (Weesner 1965, Nutting 1990).

[^{14}C]Fipronil. BASF Corp. (Research Triangle Park, NC) provided [^{14}C]fipronil {5-amino-1-[2,6-dichloro-4-(trifluoromethyl)phenyl]-4-[(trifluoromethyl)sulfinyl]-1*H*-pyrazole-3-carbonitrile} in toluene (CAS no. 120068-37-3). The specific activity was 77.03 μCi/mg with a purity of 99.4% (batch no. 849-0101).

Soil Preparation and Treatment with [^{14}C]Fipronil. Soil was collected in Lancaster Co., NE, and it was autoclaved in a Napco model 8000 DSE Autoclave (Napco Scientific Co., Tualatin, OR) for 2 h at 110°C. Autoclaved soil was dried under a fume hood for >24 h and stored in plastic containers (46 by 60 by 38 cm) (Cornerstone products, Houston, TX) until experimental use. Analysis of soil characteristics was conducted by the Soil and Plant Analytical Laboratory at the University of Nebraska-Lincoln. The soil was identified as a silty clay loam (16% sand, 47% silt, and 37% clay) with an organic matter (OM) content of 2.6% and a pH of 6.8. Soil was treated to a concentration of 1 ppm (micrograms per gram) [^{14}C]fipronil in acetone. Treated soils were then dried under a fume hood for 12 h to allow for evaporation of the acetone.

Fipronil Uptake Experiment. In total, 20 petri dishes with fipronil-treated soil were used to treat termites. Each petri dish (15 by 100 mm; Kord-Valmark Labware, Brampton, ON, Canada) was provisioned with 15 g of 1 ppm [^{14}C]fipronil (1.14 μCi)-treated soil moistened with 4.5 ml of distilled water. One-hundred fifty third-fifth instars of *R. flavipes* workers were placed in each petri dish. The experimental design was a randomized complete block design with four replications per temperature. A total of 20 environmental growth chambers were used to test five temperature regimes (12, 17, 22, 27, and 32°C). One petri dish was placed in each growth chamber held in complete darkness. Five termites were sampled from each unit at 0, 1, 2, 4, 6, 8, 12, and 24-h intervals to determine [^{14}C]fipronil uptake from treated soil.

Termite Staining. Five plastic containers (6.5 by 17.5 cm; Tristate Plastic Inc., Dixon, KY) were provisioned with 180 g of washed sand and moistened with 50 ml of distilled water. Nile blue-A formulated in methanol was applied to qualitative Whatman filter papers (Whatman, Kent, United Kingdom) to achieve a 0.1% concentration (wt:wt). The filter papers were then dried under a fume hood and subsequently placed in each container. Five hundred, third–fifth instar termite workers were placed in each of the five containers previously acclimated for 24 h to one of the temperatures to be tested (12, 17, 22, 27, or 32°C) and allowed to feed on stained filter papers for 120 h resulting in a visible blue coloration of termite tissues.

Fipronil Transfer Experiment. In total, 60 test chambers were used for the transfer experiment. Test chambers consisted of a soil chamber and a food chamber connected via polyethylene tubing to facilitate contact between donors and recipients while termites moved between chambers. The soil chamber consisted of a petri dish (15 by 100 mm; Kord-Valmark Labware) provisioned with 15 g of autoclaved soil moistened with 4.5 ml of distilled water. The food chamber was constructed from a petri dish (10 by 35 mm, BD Biosciences, San Jose, CA) provisioned with a piece of white pine (*Pinus* spp.) (2 by 1 by 0.5 cm) soaked overnight in distilled water. Soil and food chambers were connected by a 5-cm piece of polyethylene tubing (4.3-mm internal diameter; Watts Regulator Co., North Andover, MA). The tubing was secured to each chamber using a hot glue gun.

Donor termites were stained blue as described above. Three hundred donor termites were maintained on 1 ppm [^{14}C]fipronil-treated soil in a 15- by 100-mm petri dish held at one of five temperatures (12, 17, 22, 27, or 32°C) for 2 h. Recipient termites in the proper ratio were placed in each of the test chambers described above. Test chambers were randomly assigned to growth chambers at a particular temperature where they were held for 2 h. After 2 h, donor termites were combined with recipient termites at ratios of 1D:5R, 1D:10R, and 1D:20R each containing 35, 19, and 10 donor termites, respectively. The experimental design was a 4 by 3 factorial (five temperatures by three termite ratios) with four replicates per treatment. Recipient termites were sampled at 0, 6, 12, 24, 48, and 72 h to evaluate the transfer of [^{14}C]fipronil.

Radioactivity Sampling Procedure. Random samples of termites were collected at each interval from every replicate for uptake (five termites) and transfer (10 recipients) experiments. The termites were placed in a 7 ml scintillation vial (VWR Int., Inc., Chicago, IL). Three hundred microliters of Soluene-350 tissue solubilizer (PerkinElmer Life and Analytical Sciences, Boston, MA) was added to each vial. The

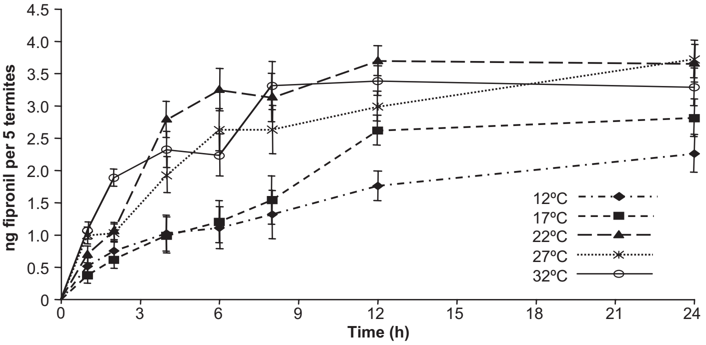

Fig. 1. Uptake of [^{14}C]fipronil by *R. flavipes* workers from soil treated at a 1 ppm concentration at five different temperatures. Bars indicate standard error of the mean.

vials were then held at 50°C for 1 h to speed the rate of tissue digestion. After digestion, 5 ml of Ecolite$^+$ scintillation cocktail (MP Biomedicals, Aurora, OH) was added to each vial, and radioactivity was measured using a 1209 Rackbeta liquid scintillation counter (LKB Wallac Inc., Gaithersburg, MD). Data were converted to disintegrations per minute from which the amount (nanograms) of [^{14}C]fipronil was calculated assuming that all radioactivity was associated with fipronil.

Statistical Analysis. Amounts of [^{14}C]fipronil were compared by analysis of variance (ANOVA) by using the PROC MIXED procedure (SAS Institute 2003) to detect differences between temperatures and ratios at $\alpha = 0.05$. Means were separated using Fisher least significant difference (LSD) procedure ($P \leq 0.05$).

Results

For the purposes of our study, we assume that all radioactivities are associated with the parent fipronil compound. We did not purify the samples before measuring radioactivity. It is possible that some of the radioactivity that was measured was associated with fipronil metabolites or other degradation components. We attempted to limit the time of fipronil exposure in the soil before experiment initiation to lessen the degree of degradation.

[^{14}C]Fipronil Uptake. Temperature significantly influenced uptake of [^{14}C]fipronil by termites from soil at all sampling intervals tested: 1 h ($F = 5.17$; df = 4, 15; $P = 0.0018$), 2 h ($F = 13.52$; df = 4, 15; $P < 0.0001$), 4 h ($F = 8.23$; df = 4, 15; $P = 0.0010$), 6 h ($F = 8.11$; df = 4, 15; $P = 0.0011$), 8 h ($F = 6.06$; df = 4, 15; $P = 0.0041$), 12 h ($F = 10.99$; df = 4, 15; $P = 0.0002$), and 24 h ($F = 4.22$; df = 4, 15; $P = 0.0173$) (Fig. 1). The initial rate of uptake is important as termites generally foraging in and out of treated zones. The rates of uptake ($\leq$2 h, per termite) in our study were as follows: 12°C ($y = 0.0754x + 0.0093$, $R^2 = 0.9561$), 17°C ($y = 0.0616x + 0.0045$, $R^2 = 0.9845$), 22°C ($y = 0.0.1077x + 0.0115$, $R^2 = 0.9669$), 27°C ($y = 0.1029x + 0.0324$, $R^2 = 0.7711$), and 32°C ($y = 0.1884x + 0.0083$, $R^2 = 0.9943$).

A greater amount of [^{14}C]fipronil was acquired by termites held at temperatures $\geq$22°C (Fig. 2). At 4, 6, and 8 h, there was no significant difference between temperatures 12 and 17°C: 4 h ($t = 0.09$, df = 15, $P = 0.9306$), 6 h ($t = 0.20$, df = 15, $P = 0.8435$), and 8 h ($t = 0.41$, df = 15, $P = 0.6858$). However, by 12 h we observed a separation in the amount of fipronil acquired by termites held at 12 and 17°C ($t = 2.67$, df = 15, $P = 0.0175$). By 24 h, we had a characteristic uptake profile based on temperature. Numerically, the highest level of [^{14}C]fipronil uptake occurred at 22–32°C followed by moderate uptake at 17°C and still lower at 12°C.

The onset of a symptomatic response to fipronil was observed in our experiment starting at 8 h in units held at 27 and 32°C, by 12 h in units held at 17–22°C, and all units by 24 h. The onset of intoxication in termites may have reduced their behavior and activity thus slowing continued uptake. This trend was evident in the data (Fig. 1) with the exception of 27°C where data indicated a $\approx$20% increase in [^{14}C]fipronil present in termites between 12 and 24 h. The observed onset of a symptomatic response to fipronil by termites agrees with observations by Saran and Rust (2007). They reported that termites exposed to 1 ppm fipronil for 1 h showed considerable changes in their ability to transverse pheromone trails over 8 h.

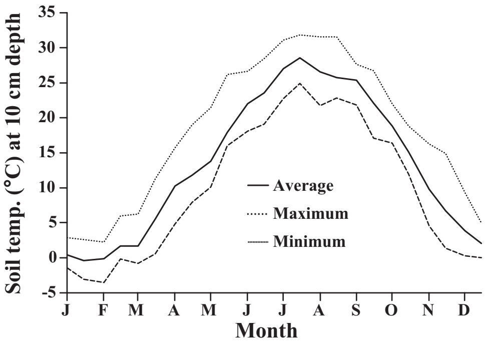

Fig. 2. Average daily soil temperatures (Celsius) from 1995 to 2005 at 10-cm depth in Lincoln, NE.

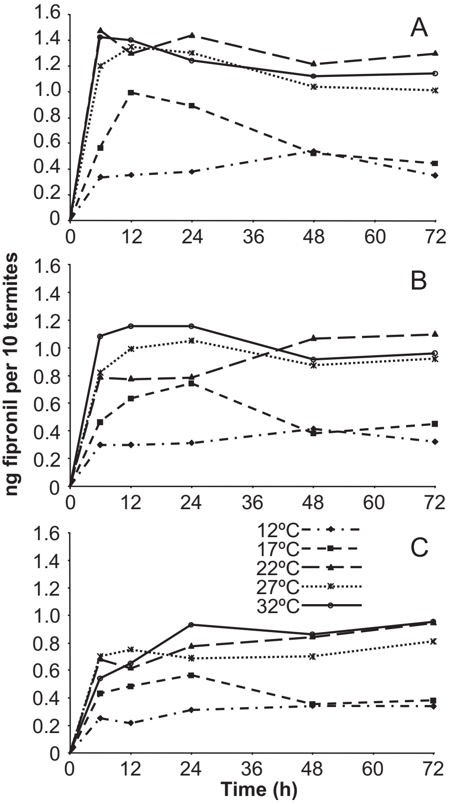

Fig. 3. Horizontal transfer of [^{14}C]fipronil at ratios of (A) 1:5, (B) 1:10, and (C) 1:20 donor to recipient *R. flavipes* at five different temperatures.

We extrapolate from our data that as soil temperatures decrease in fall and winter the rate of fipronil acquisition from treated soils by termites also likely decreases. Average daily soil temperatures (Celsius, 10 cm in depth) in Lincoln, NE, from 1995 to 2005 were collected by the High Plains Regional Climate Center at the University of Nebraska, Lincoln (Fig. 2). The average high soil temperature for the year occurred during July (28.5°C) and the low during January (−0.4°C). The temperatures selected for our laboratory experiment are meant simulate field temperatures termites may encounter while foraging, in Nebraska, between the months of April to November.

[^{14}C]Fipronil Transfer. Our data clearly indicate that [^{14}C]fipronil was transferred at all donor-to-recipient ratios and ambient temperatures tested within the range of 12–32°C. There was no significant temperature by ratio interaction at 6 h ($F = 0.66$; df = 8, 27; $P = 0.7239$), 12 h ($F = 1.12$; df = 8, 25; $P = 0.3853$), 24 h ($F = 0.87$; df = 8, 29; $P = 0.5555$), 48 h ($F = 0.07$; df = 8, 28; $P = 0.9997$), or 72 h ($F = 0.29$; df = 8, 27; $P = 0.9634$).

In all ratios and at all temperatures the majority of fipronil was transferred within the first 6 h of donor and recipient termite interaction (Fig. 3). In the 1D:5R ratios, maximal transfer occurred within 6 h from 17 to 32°C. At 12°C, the lowest temperature tested, the greatest increase in [^{14}C]fipronil transfer also occurred within 6 h of initial interaction. However, the amount of fipronil transferred was ≈62% of the maximal amount achieved by 48 h. At lower ratios (1D:10R and 1D:20R), similar results were seen although maximal rates of transfer were not achieved as quickly.

Within the 1D:10R ratio, the amount of [^{14}C]fipronil transferred in the first 6 h varied with temperature. At 32°C transferred toxicant in the first 6 h was ≈94% of the maximal amount transferred by 12 h. At 27°C, ≈74% transferred (≤ 6 h) with maximal transfer occurring by 24 h, 22°C ≈74% transferred (≤ 6 h) with maximal transfer by 48 h, 17°C ≈62% transferred (≤ 6 h) with maximal transfer by 24 h, and 12°C ≈79% transferred (≤ 6 h) with maximal transfer by 72 h.

Similar results occurred within the 1D:20R ratio (Fig. 3). At 32°C, ≈95% transferred (≤ 6 h) with maximal transfer by 72 h, 27°C ≈81% transferred (≤ 6 h) with maximal transfer by 72 h, 22°C ≈73% transferred (≤ 6 h) with maximal transfer by 72 h, 17°C ≈78% transferred (≤ 6 h) with maximal transfer by 24 h, and 12°C ≈74% transferred (≤ 6 h) with maximal transfer by 48 h. These data show the rapid initial transfer of [^{14}C]fipronil followed by a steady increase in transferred amount thereafter. However, at 17°C maximal transfer occurred by 24 h, which does deviate from this pattern.

[^{14}C]Fipronil was detected on or in recipient termites in the following sequence 1D:5R > 1D:10R > 1D:20R. Statistical analysis indicated, independent of temperature, that significant differences in fipronil acquisition by recipient termites occurred at 6 h ($F = 4.44$; df = 2, 27; $P = 0.0215$), 12 h ($F = 20.95$; df = 2, 25; $P < 0.0001$), and 24 h ($F = 7.52$; df = 2, 29; $P = 0.0023$). Within the 6- and 24-h sampling intervals, the 1D:5R ratio had a higher amount of transferred [^{14}C]fipronil than the 1D:10R and 1D:20R ratios, whereas the later two ratios did not differ ($P > 0.05$). At 12 h, all ratios were significantly different from each other ($P < 0.05$). Most efficient transfer (0.148 ± 0.057 ng per receiving termite) occurred at 6 h in the 1D:5R ratio when held at 22°C.

Discussion

Three major points were revealed in the data: 1) a majority of fipronil transfer occurred in the first 6 h of donor and recipient interaction, 2) temperature affected transfer in the first 6 h as there was a positive relationship, and 3) transfer was influenced by ratio 1D:5R > 1D:10R > 1D:20R. The effect of temperature on the uptake and transfer of fipronil may be multifaceted. Lower temperatures reduce termite behaviors such as foraging, feeding, and generally make termites lethargic, thus suppressing activities that result in uptake and transfer. A decreased physiological response of termites may occur because of slower movement of a toxicant to its target site. In addition, temperature itself can have a detrimental effect on survivorship of termites.

Our selected temperature regime for this study was 12–32°C with the two highest temperatures being 27 and 32°C. The median temperature tested was 22°C, which is close to the temperature of 21.1°C where maximum feeding activity in *R. hesperus* was reported by Smith and Rust (1993b). The 27°C temperature regime may have been high enough to cause greater mortality in *R. flavipes* based on temperature alone, and the 32.2°C regime would likely cause greater mortality if termites were held at this temperature for an extended period. Additionally, the current study only examined the short-term ($\leq$24-h) uptake profile with termites held in constant contact with treated soils. Extended contact with treated soils is unlikely under field conditions because termites generally forage in and out of treated zones. However, the nonrepellent nature of fipronil would not prevent extended contact from occurring.

Critical temperatures for *R. flavipes* were reported by Hu and Appel (2004). The researchers reported mean monthly CTMax values ranging from 43.6 to 44.9°C, much higher than normal soil temperatures in most of United States. The CTMin values were between 1.5 and 4.8°C, indicating the temperatures where we would expect foraging to cease and thus fipronil uptake. The effects of temperature (15.6–32.2°C) and humidity (30–100%) on the survivorship of *R. hesperus* have been reported previously (Smith and Rust 1993a). Their results indicated that survival was greatest at high humidity and lower temperatures. The effect of temperature (15.6–32.2°C) on tunneling distances, feeding, and oxygen consumption also has been reported (Smith and Rust 1993b). They found that tunneling rates at 15.6°C were less than at higher temperatures tested. A decrease in tunneling rate could explain the lower level of uptake reported in the current study. Because fipronil is a contact insecticide, a decrease in tunneling should have a positive relationship with uptake.

Subterranean termite seasonal foraging activity in the soil is affected by temperature fluctuations (Haverty et al. 1974, 1999; Jones et al. 1987; Haagsma and Rust 1995; Houseman et al. 2001). Field data indicate that foraging termites move deeper into the ground during colder temperatures and remain below the freezing zone (Esenther 1969), and they may retreat to depths >100 cm during winter (Cabrera and Kamble 2001). Soil temperature data, at 10-cm depth, in Lincoln, NE, rises above 23°C in June and falls below this temperature in September (HPRCC 2005). *Reticulitermes hageni* Banks and *R. flavipes* occurrence in ground monitoring stations is affected by temperature and moisture where *R. hageni* was most prevalent when temperatures rose above 23°C and soil moisture decreased below 30% (Houseman et al. 2001). In contrast, *R. flavipes* occurrence was greatest when soil moisture was above 25% and temperatures fell below 23°C. Extrapolating from the results of Houseman et al. (2001) *R. flavipes* may limit foraging in this soil zone during this time and seek soil surfaces lower in temperature or retreat to deeper soil. However, although *R. flavipes* may prefer cooler soils, we have been able to readily collect termites in the field during peak temperature months in the top 10 cm of soil.

A reduction in motor coordination was observed when termites reached temperatures between 5 and 6°C (Hu and Appel 2004). Strack and Myles (1997) observed that *R. flavipes* continued to forage as temperature dropped from 20 to 10°C as we observed in our study, foraging reduced as temperatures dropped from 10 to 5°C, and none took place at 0°C.

In the current study, termites were monitored for 1 d (uptake experiment) and 3 d (transfer experiment). Approximately 25% *R. hesperus* mortality was reported at 3 d when held at 32.2°C (Smith and Rust 1993b). The top 10 cm of soil will approach the higher temperature regimes selected for this study during summer in Nebraska. Termites do forage in the top 10 cm of soil during these times, but they may periodically retreat to soil depths with lower temperatures, thus allowing them to recover and avoid the higher mortality rates. This adds a dynamic to the relationship between uptake and transfer based on temperature. Termites may acquire fipronil at higher soil depth with warmer temperatures, and, if able to retreat to depth before a symptomatic response, transfer may occur at a lower temperature resulting in less efficient transfer of toxicant if the soil is below 22°C.

Results from Spomer and Kamble (2006) showed an increase in [^{14}C]noviflumuron uptake as a result of greater activity and feeding at higher temperatures. Results in that study indicated that the feeding uptake profile was similar when temperatures were 19–27°C within the first 72 h. Uptake of [^{14}C]fipronil in the current study showed similar trends to noviflumuron uptake at sampling intervals 4, 6, and 8 h.

In general, termite activity increases as temperatures rise. Higher biological activity of termites at increased temperatures likely resulted in a substantial increase in [^{14}C]fipronil uptake. Results confirm the fipronil uptake profile reported by Saran and Rust (2007) at 1 ppm and 24-h sampling interval. If any, slight uptake variations may be due to greater soil binding of fipronil to the soil used in our study. We used a soil with 2.6% OM, whereas their study used sand that generally has a lower OM content. Fipronil, with a K_{oc} value of 727 (Tingle et al. 2003), may have been bound tighter to the larger organic fraction in the substrate we used resulting in slightly less uptake.

The [^{14}C]noviflumuron study indicated that uptake, transfer, clearance, and loss of toxicant in fecal material occurred at a greater rate at warmer temperatures (23–27°C) with 19°C being a median temperature and 15°C having significantly lower activity (Spomer and Kamble 2006). Similar observations were made in the current study. These data corroborate that both biological and physical behaviors involved in acquisition and transfer of chemical toxicants begin to slow below 22°C. Temperatures >15 and <22°C result in slowed activity but are still higher than that seen at 12 and $\leq$15°C.

Most toxicant was transferred in the first six hours of donor and recipient interaction. Our data agree with Saran and Rust (2007) who report maximum transfer occurring within 24 h. At the higher ratios tested (1D:10R and 1D:20R) horizontal transfer occurred but the general trend of steady increase may be attributed to the higher number of recipient termites in contrast to donor termites. At this ratio it will take longer for a toxicant to diffuse through a termite colony. Similar data trends were obtained with [^{14}C]noviflumuron transfer with identical donor and recipient ratios of *R. flavipes* (Spomer and Kamble 2006).

Colonies of *R. flavipes* can reach numbers in the hundreds of thousands, if not higher, making it difficult to predict what percentage of the foraging population may actually contact a treated soil zone. Ratios we have used may be higher than what donor to recipient ratios may be in the field. What percentage of a population exposed to a treated zone will depend on the location of the nest, size of the colony, and number and availability of food sources.

The reported LD_{50} for *R. hesperus* workers is 0.16 ng fipronil and the LD_{95} is 0.75 ng at 7 d (Rust and Saran 2006). Levels of transfer in our transfer experiment with donors exposed to 1 ppm fipronil-treated soil are below this LD_{50} value. As such, we do not expect that total mortality of the test population will occur under our conditions. Recipient termites did not receive a lethal dose of toxicant after mixing with donor *Coptotermes formosanus* Shiraki workers exposed to 1 ppm fipronil $\leq$24 h (Shelton and Grace 2003). A mortality response in recipient *C. formosanus* was only measured when donors were exposed to $>$10 ppm fipronil (Shelton and Grace 2003). Su (2005) used 80.8 ppm fipronil-treated soil to document lethal transfer over short distances ($<$5 m).

Depending on the point of termite entry into a structure (e.g., slab, basement wall, and sill plate) termites can potentially forage year round. In addition, the temperature of the soil adjacent to basement walls of heated building may possibly be higher than soils located farther away from the structure. This could potentially allow termite foraging in these areas where termiticides are applied and thus result in uptake and potential transfer of toxicant during colder months of the year. The current study shows that increased temperatures, during warmer months of the year when termite foraging is generally the greatest, may result in greater uptake and subsequently more horizontal transfer of fipronil between termites.

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