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Temperature Effect on Kinetics of Uptake, Transfer, and Clearance of [14C]Noviflumuron in Eastern Subterranean Termites (Isoptera: Rhinotermitidae)

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ABSTRACT   [14C]Noviflumuron uptake, clearance, rate of excretion, and transfer from treated to untreated termite workers were evaluated at 15, 19, 23, and 27°C. Feeding units were constructed from plastic containers provisioned with washed sand, distilled water, [14C]noviflumuron-treated feeding discs (0.05 or 0.5% [AI]), and *Reticulitermes flavipes* (Kollar) workers. Feeding units were held in environmental growth chambers preset at 15, 19, 23, and 27°C. The amount of [14C]noviflumuron present within *R. flavipes* was measured by scintillation counting and subsequently quantified. Uptake of noviflumuron by *R. flavipes* workers at 15°C was ≈2.8 times less than at 19 or 23°C and ≈4.4 times less than at 27°C. The highest uptake of [14C]noviflumuron occurred at 27°C and 144 h. Most transfer of [14C]noviflumuron from treated to untreated termite workers occurred between 19 and 27°C. [14C]Noviflumuron had a half-life in *R. flavipes* workers of ≈31–45 d, dependent on temperature. A higher amount of [14C]noviflumuron was lost through excretion at ≥19°C (≈15–22%) compared with 15°C (0.27%). Results indicated that increased uptake, transfer, and clearance of noviflumuron by *R. flavipes* occurred at warmer temperatures (19–27°C), and all of these processes were significantly lower at 15°C.

KEY WORDS   noviflumuron, *Reticulitermes flavipes*, termite bait, Sentricon Termite Colony Elimination System

TERMITE CONTROL AND REPAIR costs are estimated to be in the billions of dollars annually in the United States. Of the estimated $1.5 billion spent annually, subterranean termites are responsible for 80% of all termite damage (Su 1991). Subterranean termites offer a unique control challenge because of their cryptic behavior and hidden foraging pathways. Subterranean termites may remain hidden behind external veneers, within wall voids, within wood, and under flooring, but their foraging tubes can sometimes be very conspicuous.

Termite feeding and foraging activities are disrupted by seasonal temperature variations. Esenther (1969) provided field data indicating that termites move deeper into the ground and remain below the freezing zone during winter. Based on laboratory data, Strack and Myles (1997) and Cabrera and Kamble (2001) have extrapolated that subterranean termites may retreat to soil depths >100 cm below the soil surface and remain below the freezing zone.

Subterranean termites have been controlled by applying termiticides to the soil, creating a continuous barrier around the perimeter (Kamble et al. 1984). The advent of termite bait technology offers an alternative to conventional liquid termiticide treatments.

Esenther and Gray (1968) were the first to suggest that a slow-acting bait toxicant could be used to eliminate field colonies of subterranean termites. Since the mid-1990s several bait active ingredients ([AIs]) including hexaflumuron, sulfluramid, and diflubenzuron have been registered. Noviflumuron, a chitin synthesis inhibitor, replaced hexaflumuron in 2003 in the Sentricon Termite Colony Elimination System. Effective termite control using baits requires the active ingredient to be slow-acting and transferred from termite to termite through grooming and stomodeal and proctodeal feeding. These chemicals must be highly palatable and nondeterring to termite workers (Myles 1997).

Chitin synthesis inhibitors act by disrupting the normal molting process in termites. Death occurs because termites are unable to separate from their exuvia. For most insects, the time between molts may be extended as temperatures decrease. Mortality caused by chitin synthesis inhibitors may be delayed during colder times of year. The success of hexaflumuron in termite bait has been reported at various geographic locations against several subterranean termite species (Su 1994, DeMark et al. 1995, Tsunoda et al. 1998, Sajap et al. 2000, Prabhakaran 2001, Su 2003). However, very few publications are available on noviflumuron activity against termites (Smith et al. 2002, Karr et al. 2004).

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Data on uptake, transfer, and clearance of noviflumuron in termites at different temperatures have not been reported previously.

The use of radiolabeled compounds to characterize feeding, trophallaxis, and foraging in drywood termites has been reported by Afzal (1983a, b, 1984) who found that trophallactic rates were affected by group size, feeding potentials, and excretory differences among groups of termite colony members. Other researchers investigated trophallaxis and feeding behavior in termites by using radioactive compounds (McMahan 1963, Khan et al. 1981, Traniello et al. 1985, Rosengaus et al. 1986). Spragg and Fox (1974) and Spragg and Patton (1980) used a radioactive tracer to determine nesting systems and estimate population levels. Recently, Sheets et al. (2000) and Karr et al. (2004) used [^{14}C]hexaflumuron and [^{14}C]noviflumuron to compare uptake, transfer, and clearance rates within *R. flavipes* at a constant temperature. Noviflumuron consumption by *R. flavipes* workers and resulting mortality were determined by Van den Meiracker et al. (2005) and Spomer and Kamble (2005). The research reported here was undertaken to 1) quantify the uptake, transfer, and clearance of [^{14}C]noviflumuron in *R. flavipes* at different temperatures and 2) determine [^{14}C]noviflumuron rate of excretion by *R. flavipes.*

Materials and Methods

Termite Collection and Rearing. Termites were collected on 11 May 2004 from a log near a residential home in Lincoln, NE. The log was cut into pieces ($\approx$60 cm in length and $\approx$30 cm in diameter), placed in 125-liter garbage bags (Webster Industries, Peabody, MA), and transported to the laboratory. Termites were removed from the logs and placed in plastic containers (35 by 25 by 10 cm) provisioned with moistened sand and corrugated cardboard as a food source. Termites were allowed to acclimate at 24°C and 75% RH for $\approx$30 d before use in experiments. Taxonomic identification was confirmed using soldier morphology (Weesner 1965, Nutting 1990).

[^{14}C]Noviflumuron-Treated Feeding Discs. Radioactive technical grade noviflumuron, *N*-[[[3,5-dichloro-2-fluoro-4-(1,1,2,3,3,3-hexafluoropropoxy) phenyl] amino] carbonyl]-2,6-difluorobenzamide-difluorophenyl-Ph-UL-[^{14}C] (inventory #1881, specific activity 33.0 mCi/mmol) was provided by Dow AgroSciences LLC (Indianapolis, IN). Feeding discs ($\approx$ 6 mm in diameter) of Whatman filter papers (Whatman, Kent, United Kingdom) were cut with a hand-held single-hole paper punch. Four groups of 100 discs were used to calculate the dry weight of each disc (2.60 mg).

Feeding discs used for the transfer experiment were stained with Nile blue-A formulated in acetone solution to achieve a 0.1% concentration (wt:wt). After staining each feeding disc, they were treated with [^{14}C]noviflumuron and allowed to air dry for 15 h. Each feeding disc used in uptake and clearance experiments was treated with 0.077 μCi of technical grade [^{14}C]noviflumuron in acetone solution (wt:wt) by using a 25-μl syringe (Hamilton Co., Reno, NV). Feeding discs were treated with 0.765 μCi of [^{14}C]noviflumuron (wt:wt) per disc for the transfer and rate of excretion experiments.

General Termite Sampling Procedure for Radioactivity. Random samples of five termites were taken at each sampling interval for each replication in all experiments. Five termites from each sample were placed in a 7-ml glass scintillation vial. Two-hundred microliters of Soluene-350 tissue solubilizer (Perkin-Elmer Life and Analytical Sciences, Boston, MA) was added to each vial. The vials containing the termites and tissue solubilizer were heated at 50°C for 1 h to speed the rate of tissue digestion. Five milliliters of Ecolite+ scintillation cocktail (MP Biomedicals, Aurora, OH) was added to each scintillation vial after tissue digestion. Radioactivity was measured using a 1209 Rackbeta liquid scintillation counter (LKB Wallac Inc., Gaithersburg, MD). Output data were converted to disintegrations per minute (dpm) from which average noviflumuron amounts (nanograms) per termite were calculated.

Uptake of [^{14}C]Noviflumuron in *R. flavipes* at Different Temperatures. Sixteen feeding units were used in this experiment. Each feeding unit was constructed using a plastic container (6.5 cm in height by 17.5 cm in diameter, Tristate Plastic Inc., Dixon, KY) and contained 190 g of distilled water-washed and oven-dried sand subsequently moistened with 40 ml of distilled water. Each feeding unit was provisioned with 200 termite workers (third–fifth instars) and untreated filter paper discs as a food source. Four feeding units were placed in each growth chamber set at 15, 19, 23, or 27°C in continuous scotophase and allowed to acclimate for 72 h. After the acclimation period, the untreated filter paper discs were removed and replaced with 20 filter paper discs treated with 0.077 μCi of [^{14}C]noviflumuron per disc and feeding units returned to their preassigned growth chamber. Termites were allowed to feed on the treated filter papers for 72 h, which were then replaced with untreated filter paper discs as a food source. The experimental design was a randomized complete block design, blocked by temperature with four replications per temperature treatment. Termites were sampled from each feeding unit at 0-, 24-, 72-, 144-, and 288-h intervals to determine [^{14}C]noviflumuron uptake.

Transfer of [^{14}C]Noviflumuron from Treated to Untreated Termite Workers at Different Temperatures. Four groups of 400 termite workers (third–fifth instars) were placed in feeding units as described above. Each feeding unit received 105 Nile blue-A stained, [^{14}C]noviflumuron (0.765 μCi)-treated feeding discs. Feeding units were placed in growth chambers set at 15, 19, 23, or 27°C in continuous scotophase, and termites were allowed to feed for 7 d. Termite workers were irreversibly stained blue as a result of feeding on stained feeding discs and referred to as "blue." Four groups of $\approx$2,500 untreated termite workers, referred to as "white," were starved for 24 h before combining with blue termites at ratios of 1 blue:5

white, 1 blue:10 white, and 1 blue:20 white. Feeding units contained a combined total of 210 termites. One feeding unit from each ratio was randomly placed in one of four growth chambers of similar temperature experienced by the original termite group. The experimental design was a 4 by 3 factorial (four temperatures by three termite ratios) with four replications per treatment. White termites were sampled at 0, 8, 24, 48, 96, 168, 240 h to evaluate transfer of [^{14}C]noviflumuron.

Clearance of [^{14}C]Noviflumuron from *R. flavipes* at Different Temperatures. Six hundred *R. flavipes* workers (third–fifth instars) were placed in each of four feeding units containing 150 feeding discs treated with 0.077 μCi of [^{14}C]noviflumuron per disc. Each feeding unit was placed in a growth chamber set at 15, 19, 23, or 27°C in continuous scotophase and held for 7 d. On day 7, termites from each feeding unit were subdivided into four groups of 150 termites and placed into individual feeding units. Each of these units was provided with untreated filter paper discs as a food source.

In total, 16 feeding units were constructed. The experimental design was a randomized complete block, blocked by temperature with four replications per temperature. Sampling began when groups of termites were segregated into smaller groups on day 7. The sampling intervals were 0, 16, 32, 48, 72, 144, and 240 h.

[^{14}C]Noviflumuron Rate of Excretion. Thirty *R. flavipes* workers (third–fifth instars) were placed in each of 16 feeding units containing five feeding discs treated with 0.765 μCi of [^{14}C]noviflumuron per disc. Four feeding units were placed in each of four growth chambers set at 15, 19, 23, or 27°C in continuous scotophase. Termites were allowed to feed on treated discs for 5 d. On day 5 of [^{14}C]noviflumuron feeding, two samples of five termites were taken from each feeding unit and placed in separate 7-ml scintillation vials. The samples were referred to as sample A and sample B. Sample A provided the baseline amount of [^{14}C]noviflumuron present in the termites at each temperature determined through scintillation counting. Moistened untreated filter paper feeding discs were added to B samples, which were returned to their originally assigned growth chambers. At 24 h, termites from sample B were removed and placed in a new scintillation vial referred to as sample C. The sample B vials with the remaining feeding disc and excrement were evaluated for [^{14}C]noviflumuron presence through scintillation counting. Sample C termites also were evaluated for loss of fecal material at 24 h.

The experimental design was a randomized complete block design, blocked by temperature with four replications per temperature. Data were compared to determine the effect of temperature on [^{14}C]noviflumuron loss through excretion at 24 h.

Statistical Analysis. Amounts of [^{14}C]noviflumuron per termite were examined by analysis of variance (ANOVA) by using the PROC MIXED procedure (SAS Institute 2001) to detect significant differences at α = 0.05. Means were separated using Fishers's least
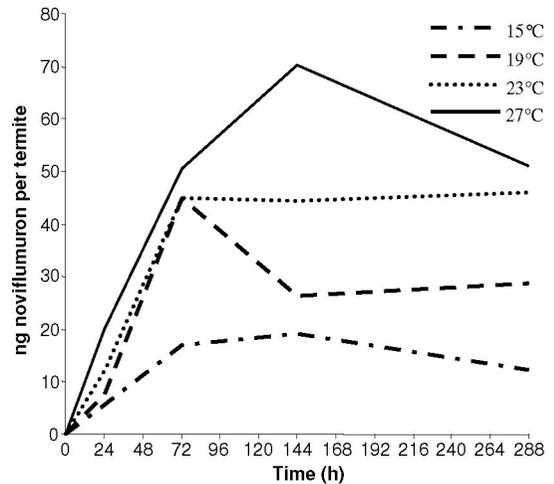
Fig. 1. [^{14}C]Noviflumuron uptake over time in *R. flavipes* workers at four different temperatures.

significant difference (LSD) procedure ($P \leq 0.05$). Differences between means were evaluated with paired *t*-tests (SAS Institute 2001).

Results and Discussion

Uptake of [^{14}C]Noviflumuron by *R. flavipes* at Different Temperatures and Time Intervals. During the first 24 h of bait feeding no statistical difference in [^{14}C]noviflumuron amounts per termite were detected ($F = 2.97$; df = 3, 12; $P = 0.0743$). However, numerical data indicated that a greater level of uptake occurred at 27°C (19.81 ± 3.70 ng) followed decreasingly by 23°C (11.86 ± 3.70 ng), 19°C (7.32 ± 3.70 ng), and 15°C (5.49 ± 3.70 ng) (Fig. 1).

Rates of [^{14}C]noviflumuron uptake were significantly different between the various temperatures tested at the 72-h time interval ($F = 5.39$; df = 3, 12; $P = 0.0139$). Data indicated significantly less uptake occurred at 15°C compared with 19°C ($t = 3.03$, df = 12, $P = 0.0105$), 23°C ($t = 3.02$, df = 12, $P = 0.0106$), and 27°C ($t = 3.64$, df = 12, $P = 0.0034$). No difference in the amount of [^{14}C]noviflumuron taken up by termites occurred among 19, 23, and 27°C ($P > 0.05$). These results indicate a very similar (active ingredient) uptake profile at $\geq$19°C in the first 72 h, suggesting similar feeding rates at these temperatures. Under field conditions subterranean termites may feed on a variety of cellulose food sources, including baits treated with active ingredient. To accurately reflect discontinuous termite feeding at a particular location, treated feeding discs were replaced with untreated feeding discs at 72 h.

Differences in the amount of [^{14}C]noviflumuron within each termite worker occurred at 144 h ($F = 6.60$; df = 3, 12; $P = 0.0070$). [^{14}C]noviflumuron levels at 15°C were similar to those at 19°C ($t = 0.57$, df = 12, $P = 0.5767$) and 23°C ($t = 2.03$, df = 12, $P = 0.0649$), but they were significantly less at 27°C ($t = 4.07$, df =

12, $P < 0.0015$). Uptake of [14C]noviflumuron at 19 was similar to that at 23°C ($t = 1.46$, df = 12, $P = 0.1706$), but it was significantly less at 27°C ($t = 3.50$, df = 12, $P < 0.0044$). No significant differences were observed in uptake of [14C]noviflumuron at 23 and 27°C ($t = 2.04$, df = 12, $P = 0.0637$) (Fig. 1). Contrary to expectations, the amount of [14C]noviflumuron per termite continued to increase within the 27°C temperature treatment, despite the removal of treated feeding discs at 72 h. This unexpected result may be the consequence of an underestimated [14C]noviflumuron level at 72 h or the occurrence of cannibalism resulting in increased active ingredient levels.

Differences in the amount [14C]noviflumuron per termite were detected between temperature treatments at 288 h ($F = 16.25$; df = 3, 12; $P < 0.0001$). Less [14C]noviflumuron (nanograms per termite) was present at 15 than at 19°C ($t = 2.65$, df = 12, $P = 0.0212$), 23°C ($t = 5.44$, df = 12, $P = 0.0001$), and 27°C ($t = 6.27$, df = 12, $P < 0.0001$). Uptake at 19°C was significantly lower than at 23°C ($t = 2.80$, df = 12, $P = 0.0162$), and 27°C ($t = 3.62$, df = 12, $P = 0.0035$). No significant difference in [14C]noviflumuron levels was detected between 23 and 27°C ($t = 0.82$, df = 12, $P = 0.4276$) (Fig. 1).

Figure 1 indicates the highest uptake of 0.05% [14C]noviflumuron was between 72 and 288 h. [14C]noviflumuron was taken up faster by *R. flavipes* workers at warmer temperatures ($\geq$19°C). The maximum uptake of [14C]noviflumuron occurred at 27°C (70.06 ± 8.85 ng) followed by 23°C (45.86 ± 6.48 ng), 19°C (44.89 ± 6.48 ng), and 15°C (19.06 ± 8.85 ng). These results confirm expectations that more bait will be ingested by *R. flavipes* workers as temperatures increase.

Our results indicate that less noviflumuron is ingested at lower temperatures. For example, at 15°C ≈4.4 times less noviflumuron is taken up than at 27°C and ≈2.8 times less than at 19 or 23°C. Uptake of noviflumuron was ≈1.5 times less at 19–23°C than at a warmer temperature of 27°C in *R. flavipes* workers. Our results report the amount of noviflumuron present in termite bodies after feeding at a specific temperature and amount of time. Future research establishing a lethal baseline amount of noviflumuron required at molt will help to further establish an effective temperature range for noviflumuron toxicity.

Karr et al. (2004) report that peak uptake of 0.5% [14C]noviflumuron was 150 ng per termite at 360 h. Differences in amount of uptake and time of peak uptake are possibly a result of different concentrations of active ingredient used in each study (0.05 versus 0.5%).

Transfer of [14C]Noviflumuron from Treated to Untreated *R. flavipes* at Different Temperatures and Time Intervals. Noviflumuron was transferred from treated blue termites to untreated white termites at all temperatures tested. Within the range 15–27°C, termite activity is high enough to measure the trophallactic behavior of donor and receiving termites indicated in Fig. 2. Using a factorial design with temperature and ratio as treatment factors, the data
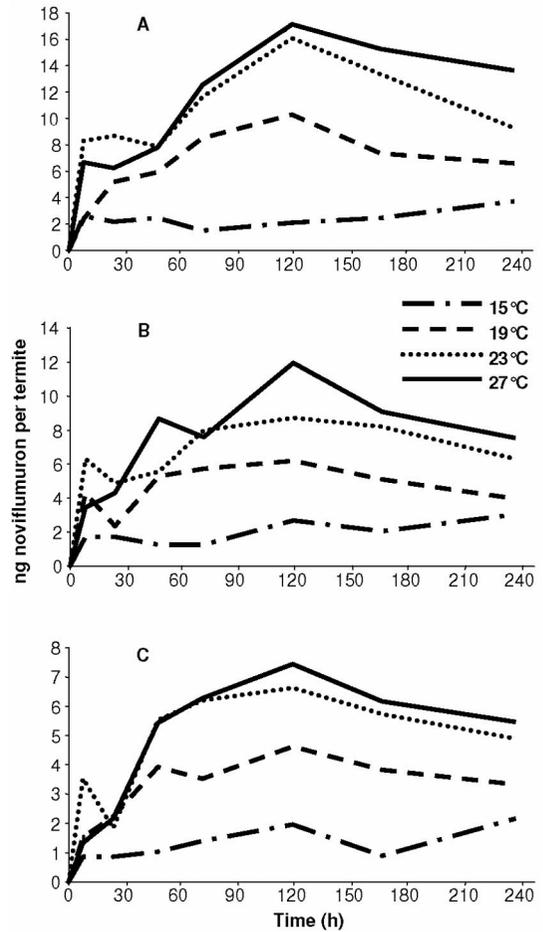
Fig. 2. Transfer of [14C]noviflumuron at ratios of (A) 1:5, (B) 1:10, and (C) 1:20 noviflumuron-treated to -untreated *R. flavipes* workers at four different temperatures.

were examined for any interactions between the two factors. Statistical analysis indicated that no significant differences were observed between treatments at 8 h ($F = 2.12$; df = 6, 24; $P = 0.0886$), 24 h ($F = 0.91$; df = 6, 24; $P = 0.5015$), 48 h ($F = 0.33$; df = 6, 24; $P = 0.9163$), 72 h ($F = 1.30$; df = 6, 24; $P = 0.2933$), and 240 h ($F = 1.46$; df = 6, 24; $P = 0.2350$) sampling intervals. However, a small peak of [14C]noviflumuron was measured in untreated "W" *R. flavipes* workers at 8 h (Fig. 2) and became more distinct at higher temperatures (23 and 27°C).

Karr et al. (2004) found that transfer of noviflumuron from treated to untreated termites peaked at 8 h in all ratios and dropped quickly thereafter. In our study, the amount of [14C]noviflumuron transferred to untreated recipient termites increased steadily after the small 8-h peak until maximum transfer occurring at the 120-h sampling interval and at 27°C. The discrepancy between data obtained in the two studies may be the result of differences in feeding unit design and temperatures used in each study.

A significant difference between treatments was detected in the 120- ($F = 5.34$; df = 6, 24; $P < 0.0133$) and 168-h ($F = 2.68$; df = 6, 24; $P = 0.0392$) sampling intervals. The data indicates that within the 1:5 and 1:10 ratios of treated to untreated termites a distinct temperature pattern emerged. A higher amount [^{14}C]noviflumuron transfer occurred at 23 and 27°C followed decreasingly by 19 and then 15°C. At the 1:20 ratio, a lower amount of noviflumuron was transferred decreasing our ability to detect differences. However, a trend did emerge: temperatures ≥19°C consistently had a greater rate of active ingredient transfer.

A small peak of [^{14}C]noviflumuron was found at 8 h in untreated termites, followed by a steady increase in transfer of noviflumuron. In general, maximum noviflumuron amounts per untreated termite worker were detected at 120 h (Fig. 2). Lowest transfer occurred at 15°C, although an increase in noviflumuron per untreated worker indicated that trophallactic exchanges were still occurring. These results confirm expectations that less trophallactic activity occurred at lower temperatures. Spomer and Kamble (2005) report that at 27°C, a 1:20 ratio of treated to untreated termites has the potential to kill a majority of the population (>85%) within 8 wk, indicating that temperature, and not ratio, likely has a more dramatic effect on toxicity. In general, the transfer data show that greater amounts of noviflumuron were transferred from treated to untreated termites at the higher temperatures. This can result from different factors. First, more noviflumuron is consumed at higher temperatures, thus making more active ingredient available to transfer. Second, termites are more active at higher temperatures, resulting in increased trophallactic exchanges. Finally, at higher ratios of treated to untreated workers more dilution of noviflumuron may occur within a population.

Clearance of [^{14}C]Noviflumuron from *R. flavipes* at Different Temperatures and Time Intervals. Differences between temperatures were significant at all temperature intervals (0, 16, 32, 48, 72, 144, and 240 h) ($P < 0.01$). Trends in data were similar throughout all observation intervals in this study. The linear trend in clearance of noviflumuron from *R. flavipes* workers is illustrated (Fig. 3). No interaction between time and temperature indicate that the slopes for each temperature trend line are not significantly different over time ($F = 1.23$; df = 18, 72; $P = 0.2640$).

Karr et al. (2004) estimated that the half-life of [^{14}C]noviflumuron in *R. flavipes* bodies to be ≈29 d. Our data were plotted into Excel graphing software (Microsoft 2002), and trend lines were fitted to the data for each temperature. The slope and intercept of the regression equations were used to estimate clearance of $-0.1185x$ at 27°C and $-0.026x$ at 15°C. Based on calculations, we estimate the half-life of noviflumuron in termite bodies to be between ≈31 and 45 d, depending on temperature within the range of 15–27°C. Long retention time of noviflumuron within termite bodies is important for the success of noviflumuron as a baiting product. The stadium duration between molts increases as temperatures decrease. A
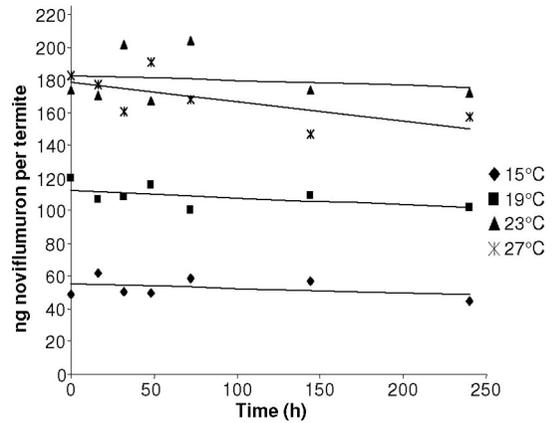
Fig. 3. Scatter plot of [^{14}C]noviflumuron clearance from *R. flavipes* at different temperatures.

sufficient amount of noviflumuron must be present at the time of molt to disrupt an ecdysal event and cause death. If noviflumuron clears from termites too quickly and an insufficient amount of active ingredient remains, the termite may molt and survive. Based on these results, noviflumuron shows excellent residual life in *R. flavipes* workers from 15 to 27°C.

Metabolism of active ingredients to less lethal metabolites is an additional detriment to the success of baits and other termiticides. Sheets et al. (2000) reported that no significant metabolism of hexaflumuron was detected in termites conducted over 40 d. Hexaflumuron, the predecessor to noviflumuron, is similar in mode of action and structure. Although no data are published on the metabolism of noviflumuron, similar results to hexaflumuron would be expected. Metabolism also is expected to be lower at cooler temperatures as physiological processes in termites slow.

Extrapolating from results obtained in uptake and transfer experiments and expected metabolism we can hypothesize that in fall, if temperatures remain warm, a high amount of active ingredient may be ingested by foraging termite workers. As temperatures decrease in winter, below 15°C, clearance and metabolism of active ingredient is slowed greatly. As temperatures increase during spring, enough active ingredient may be retained within termite bodies to cause a lethal ecdysal event. It is then possible that noviflumuron may continue to exhibit toxicity in the spring before resuming bait station feeding.

[^{14}C]Noviflumuron Rate of Excretion at Different Temperatures and Time Intervals. The initial amount of [^{14}C]noviflumuron present in termites held at each temperature was significantly different, indicating that at warmer temperatures more bait feeding occurred ($F = 50.76$; df = 3, 12; $P < 0.0001$). Significantly less [^{14}C]noviflumuron was present within termites held at 15 compared with 19°C ($t = 2.79$, df = 12, $P = 0.0163$), 23° ($t = 10.26$, df = 12, $P < 0.0001$), and 27°C ($t = 9.50$, df = 12, $P < 0.0001$). Significantly lower noviflumuron levels were detected at 19 compared
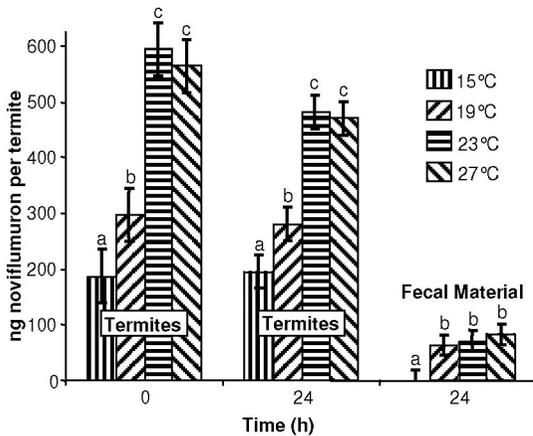
Fig. 4. Comparison of [^{14}C]noviflumuron loss in *R. flavipes* fecal material at different temperatures over 24 h. Different letters indicate significant differences ($P < 0.05$).

with 23°C ($t = 7.47$, df $= 12$, $P < 0.0001$) and 27°C ($t = 6.70$, df $= 12$, $P < 0.0001$). Data did not differ significantly between 23 and 27°C ($t = 0.77$, df $= 12$, $P = 0.4585$) (Fig. 4).

Amounts of noviflumuron remaining in termites at 24 h were similar to amounts calculated at zero time. Temperatures between 15 and 19°C ($t = 3.33$, df $= 12$, $P = 0.0060$) and 19 and 23°C ($t = 8.06$, df $= 12$, $P < 0.0001$) had significantly different amounts of [^{14}C]noviflumuron present in *R. flavipes* bodies. Temperatures 23 and 27°C were not significant from each other ($t = 0.44$, df $= 12$, $P = 0.6688$).

The amount of [^{14}C]noviflumuron measured in fecal material differed significantly based on temperature ($F = 11.90$; df $= 3, 12$; $P = 0.0007$). A lower amount of [^{14}C]noviflumuron was recovered from fecal material at 15 compared with 19°C ($t = 4.10$, df $= 12$, $P = 0.0015$), 23°C ($t = 4.59$, df $= 12$, $P = 0.0006$), and 27°C ($t = 0.92$, df $= 12$, $P = 0.0001$). No significant differences in the amount of [^{14}C]noviflumuron occurred between 19 and 23°C ($t = 0.49$, df $= 12$, $P = 0.6296$), 19 and 27°C ($t = 1.42$, df $= 12$, $P = 0.1811$), and 23 and 27°C ($t = 0.92$, df $= 12$, $P = 0.3732$) (Fig. 4).

Results indicated that noviflumuron was excreted from termite bodies and was influenced by temperature. At the 15°C, only 0.27% of [^{14}C]noviflumuron present at 24 h was lost through excretion. At ≥19°C, a higher rate of noviflumuron loss occurred in fecal material. The range of loss at these temperatures was between ≈15 and 22% in the first 24 h. Slower metabolic processes at lower temperatures may result in less excretion of active ingredient.

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References Cited

Afzal, M. 1983a. Radioisotope studies of trophallaxis in the drywood termite *Bifiditermes beesoni* (Gardner) (Isoptera). I. Effect of group size on the rate of food exchange. Mater. Org. 18: 51–63.

Afzal, M. 1983b. Radioisotope studies of trophallaxis in the drywood termite *Bifiditermes beesoni* (Gardner) (Isoptera). II. Mutual feeding potentialities of soldiers and nymphs. Mater. Organismen 18: 107–117.

Afzal, M. 1984. Radioisotope studies of trophallaxis in the drywood termite *Bifiditermes beesoni* (Gardner) (Isoptera). III. Feeding and excretory differences among grouped and isolated colony members. Mater. Org. 19: 55–67.

Cabrera, B. J., and S. T. Kamble. 2001. Effects of decreasing thermophotoperiod on the eastern subterranean termite (Isoptera: Rhinotermitidae). Environ. Entomol. 30: 166–171.

DeMark, J. J., E. P. Benson, P. A. Zungoli, and B. M. Kard. 1995. Evaluation of hexaflumuron for termite control in the Southeast U.S. Down to Earth 50: 20–26.

Esenther, G. R. 1969. Termites in Wisconsin. Ann. Entomol. Soc. Am. 62: 1274–1284.

Esenther, G. R., and D. E. Gray. 1968. Subterranean termite studies in southern Ontario. Can. Entomol. 100: 827–834.

Kamble, S. T., Gold, R. E., and J. D. Rauscher. 1984. Distribution and economic impact of subterranean termites in Nebraska. Environmental Programs Report No. 9. University of Nebraska-Lincoln.

Karr, L. A., J. J. Sheets, J. E. King, and J. E. Dripps. 2004. Laboratory performance and pharmacokinetics of the benzoylphenylurea noviflumuron in eastern subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 97: 593–600.

Khan, K. I., R. H. Jafri, and M. Ahmad. 1981. Role of trophallaxis in the dispersal of radioactive I^{131} and of bacterial infections in the termite, *Bifiditermes beesoni*. Mater. Org. 16: 189–197.

McMahan, E. 1963. A study of termite feeding relationships, using radioisotopes. Ann. Entomol. Soc. Am. 56: 74–82.

Microsoft. 2002. Microsoft Office XP. Microsoft Excel. Microsoft, Redmond, WA.

Myles, T. G. 1997. Evaluation of barium metaborate monohydrate and 2-(thiocyanomethylthio)-benzothioazole as potential bait toxicants or feeding inhibitors against subterranean termites. Proc. Entomol. Soc. Ont. 128: 99–103.

Nutting, W. L. 1990. Insecta: Isoptera, pp. 997–1032. *In* D. L. Dindal [ed.], Soil biology guide. Wiley, New York.

Prabhakaran, S. K. 2001. Eastern subterranean termite management using baits containing hexaflumuron in affected University of Iowa structures (Isoptera: Rhinotermitidae). Sociobiology 37: 221–223.

Rosengaus, R. B., J.F.A. Traniello, and C. K. Levy. 1986. Social transfer, elimination, and biological half-life of gamma-emitting radionuclides in the termite *Reticulitermes flavipes* Kol. J. Appl. Entomol. 101: 287–294.

Sajap, A. S., S. Amit, and J. Welker. 2000. Evaluation of hexaflumuron for controlling the subterranean termite *Coptotermes curvignathus* (Isoptera: Rhinotermitidae) in Malaysia. J. Econ. Entomol. 93: 429–433.

SAS Institute. 2001. SAS Institute. SAS user's guide, version 8.2. SAS Institute, Cary, NC.

Sheets, J. J., L. L. Karr, and J. E. Dripps. 2000. Kinetics of uptake, clearance, transfer and metabolism of hexaflumuron by eastern subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 93: 871–877.

Smith, M. S., L. L. Karr, J. E. King, W. N. Kline, R. J. Sbragia, J. J. Sheets, and M. Tolley. 2002. Noviflumuron activity in household and structural insect pests, pp. 345–353. *In* S. C. Jones, J. Zhai, and W. H. Robinson. [eds.], Proceedings of the 4th International Conference on Urban Pests, Charleston, SC. Pocahontas Press, Inc., Blacksburg, VA.

Spomer, N. A., and S. T. Kamble. 2005. Effect of temperature on noviflumuron performance against the eastern subterranean termite (Isoptera: Rhinotermitidae). Sociobiology 46: 335–348.

Spragg, W. T., and R. E. Fox. 1974. The use of a radioactive tracer to study the nesting system of *Mastotermes darwiniensis* Froggatt. Insectes Soc. 21: 309–316.

Spragg, W. T., and R. Patton. 1980. Tracing, trophallaxis and population measurement of colonies of subterranean termites (Isoptera) using a radioactive tracer. Ann. Entomol. Soc. Am. 73: 708–714.

Strack, B. H., and T. G. Myles. 1997. Behavioural responses of the eastern subterranean termite to falling temperatures (Isoptera: Rhinotermitidae). Proc. Entomol. Soc. Ont. 128: 13–17.

Su, N.-Y. 1991. Termites of the United States and their control. SP World 17: 12–15.

Su, N. Y. 1994. Field evaluation of a hexaflumuron bait for population suppression of subterranean termites (Isoptera: Rhinotermitidae). J. Econ. Entomol. 87: 289–397.

Su, N.-Y. 2003. Bait as a tool for population control of the Formosan subterranean termite. Sociobiology 42: 177–192.

Traniello, J.F.A., R. B. Rosengaus, and C. K. Levy. 1985. Single and double isotope labeling of social insect colonies: gamma-emitting radionuclides as individually identifiable markers. Entomol. Exp. Appl. 38: 87–92.

Tsunoda, K., H. Matsuoka, and T. Yoshimura. 1998. Colony elimination of *Reticulitermes speratus* (Isoptera: Rhinotermitidae) by bait application and the effect on foraging territory. J. Econ. Entomol. 91: 1383–1386.

Van den Meiracker, K. G., P. A. Zungoli, E. P. Benson, and W. C. Bridges, Jr. 2005. Temperature effect on survival and cellulose consumption of noviflumuron- or hexaflumuron-fed *Reticulitermes flavipes* (Isoptera: Rhinotermitidae). Sociobiology 45: 1–9.

Weesner, F. M. 1965. The termites of the United States - a handbook. The National Pest Control Association, Elizabeth, NJ.