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Effects of Decreasing Thermophotoperiod on the Eastern Subterranean Termite (Isoptera: Rhinotermitidae)

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ABSTRACT Eastern subterranean termite, *Reticulitermes flavipes* (Kollar), workers were exposed to decreasing temperatures and a shift in photoperiod to simulate conditions that are encountered during the fall in eastern Nebraska. Mean water and fat content in termites exposed to a decreasing thermophotoperiod did not differ significantly from controls or laboratory colony termites. Survival was higher for termites subjected to a decreasing thermophotoperiod than for controls. This could be attributed to acclimation to low temperatures or a delayed mortality due to lowered metabolic activity at low temperature. Previously reported data on soil temperatures taken in Lincoln, NE, at a depth of 91.4 cm showed that the temperature rarely went below 0°C. Our results, observations from previous reports of *R. flavipes* found at depths >100 cm during the winter, and previously determined lower lethal temperatures and supercooling points suggest that successfully overwintering *R. flavipes* colonies retreat to soil depths where freezing temperatures are not encountered.

KEY WORDS *Reticulitermes flavipes*, overwintering, thermoperiod, photoperiod, water content, fat content

FORAGING ACTIVITY OF the eastern subterranean termite, *Reticulitermes flavipes* (Kollar), at the soil surface gradually decreases in the midwestern and eastern United States as daily temperatures decrease during late fall. During the winter there is almost no subterranean termite activity until temperatures increase in the spring. Although there have been several studies on the overwintering biology of *R. flavipes* (Esenther 1969, Husby 1980, Davis and Kamble 1994, Strack and Myles 1997), many of the behavioral and physiological adaptations that allow this species to survive the winter in the colder parts of its range remain unknown. Strack and Myles (1997) suggested that *R. flavipes* does not enter a winter diapause although this has yet to be proven empirically. Several behaviors in termites are thought to be responses that enhance survival at cold temperatures. Greaves (1964) and Holdaway and Gay (1948) suggested that aggregation by termites within the nest is an effective means of conserving heat because it reduces the amount of exposed surface area of individual termites. They reported that temperatures within trees or mounds where termites aggregated during the winter were significantly warmer than the ambient air temperature. However, Strack and Myles (1997) found no evidence of *R. flavipes* aggregating or generating heat when exposed to cold temperatures. They concluded that *Reticulitermes* spp. do not aggregate and regulate nest temperatures like the mound-building or tree

inhabiting species because they have no centralized or main nesting site (Weesner 1970, Beard 1974, Myles and Grace 1991). Observations by Esenther (1969), Husby (1980) and Strack and Myles (1997) indicate that *R. flavipes* colonies move deep into the ground, where temperatures are higher and more uniform (Bouyoucos 1913, Mail 1930, Leather et al. 1993), and remain there throughout the winter. Because soil nesting sites are not essential, above-ground tree stumps, logs, and other large pieces of wood can also serve as refugia for *R. flavipes* during the winter because these sites provide sufficient insulation against lethal temperatures (Beard 1974, Husby 1980, Grace 1996). Snow cover also provides insulation so that temperatures in the soil or wood are above the minimum air temperature (Mail 1930, Leather et al. 1993). Heated structures and sewer systems also provide habitable overwintering sites for termites (Myles and Grace 1991).

Despite the insulatory and dampening effects of deep soil or large pieces of wood, termites and other soil and wood-dwelling insects may still be adversely affected by long-term exposure to cold. However, gradually decreasing temperatures in the weeks before winter may acclimate insects and increase their tolerance to low temperatures. Decreasing temperatures and photoperiod also serve as cues that induce behavioral, morphological and physiological responses in insects that increase their chances of surviving the winter (Leather et al. 1993). Because insect activity is lower or nonexistent during the winter, many diapausing insects increase their lipid content and rely on these reserves for survival (Leather et al.

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1993). Many insects that hibernate or diapause in the winter reduce their body water content thereby reducing the likelihood of internal ice crystal formation at freezing temperatures (Hadley 1994).

Placing a thermoperiod, or daily rise and fall of temperatures, on a photoperiod, such that the phases coincide in a natural manner, forms a thermophotoperiod consisting of a cryoscotophase and a thermophotophase (Beck 1991). Thermophotoperiods have been shown, in some cases, to elicit developmental responses in insects that would not have been predicted based on either thermo- or photoperiods alone (Beck 1991). Thus, thermophotoperiodic responses may be extremely important to the growth and development, dormancy, cold acclimation and winter survival of some insects (Beck 1991). In the fall, decreasing temperatures and daylength serve as stimuli for many organisms to prepare for winter. Insects respond by adopting various forms of dormancy including cold torpor and a physiological diapause (Beck 1991).

In this study we exposed *R. flavipes* workers to conditions that may be encountered during the weeks before the onset of winter. Our objectives were to determine if *R. flavipes* reduces its body water content and increases fat content as temperature decreases, indicating that reserves are being stored in preparation for a prolonged period of inactivity. We were also interested in finding out if a decreasing thermophotoperiod acclimates *R. flavipes* resulting in increased survival at low temperatures. Our intent is to provide additional information that, together with the results of other studies on the overwintering biology of *R. flavipes*, will provide a more comprehensive understanding of the abilities of this species to survive winter in temperate regions.

Materials and Methods

Termites. Termites were collected in Lincoln, NE, from in-ground traps consisting of covered 3.8-liter plastic buckets with the bottoms removed and containing rolls of corrugated cardboard. The termites were transferred to a 56.8-liter glass aquarium, containing a mixture of moist soil and fill sand, which was kept at $\approx 23^{\circ}\text{C}$. A large glass plate covered the top of the aquarium to reduce moisture loss and pieces of ash and pine placed on the soil surface served as a food source.

Experimental Set-up. Experimental units consisted of 237-ml canning jars filled with a mixture of 55 ml sterilized fill sand, 55 ml sterilized vermiculite, and 38 ml distilled water (Davis and Kamble 1994). Ash wood blocks (6.0 by 2.5 by 1.2 cm), which had been oven-dried at 75°C for 6 h, were pressed vertically and approximately half their length into the soil mixture. Groups of 75 workers were weighed to the nearest mg and placed in the jars. The units were placed in a growth chamber (model E-30B, Percival Scientific, Boone, IA) at 25°C and a photoperiod of 10:14 (L:D) h for a 2-wk acclimation period. Half of the jars were then removed, placed in a separate chamber, and

Table 1. Thermophotoperiod regime for *R. flavipes* workers during 6-wk (week 1–6) and 12-wk (week 1–12) experiments

Week	Thermoperiod regime ($^{\circ}\text{C}$) (thermophase:cryophase)	Photoperiod regime, h (photophase:scotophase)
1	25:20	10:14
2	20:15	10:14
3	15:10	8:16
4	10:5	8:16
5	5:0	8:16
6	0	8:16
7	5:0	8:16
8	10:5	8:16
9	15:10	8:16
10	20:15	10:14
11	25:20	10:14
12	25	10:14

subjected to decreasing temperatures in weekly 5°C increments over a 6-wk period (Table 1). The photoperiod was 10:14 (L:D) h for the first 2 wk followed by 8:16 (L:D) h for the remaining 4 wk. During each week, except for the last, the units were subjected to a daily 5°C thermoperiod coinciding with the photoperiod thus subjecting the termites to a daily thermophotoperiod. The units in the other chamber were kept at 25°C and a photoperiod of 10:14 (L:D) h the entire time and served as controls. At the end of each week, 3 units each from the experimental and control chambers were removed and the living termites were extracted from the jars, counted, and analyzed for water and fat content as described below. Each unit was considered a replicate for a total of three replicates per treatment period. The treatment period was the time from the start of the thermophotoperiod regime, i.e., the beginning of week 1, to the time the unit was removed.

Twelve-Week Study. We conducted a second experiment to examine the long-term effects of changing thermophotoperiod and extended exposure to cold temperatures on *R. flavipes* workers. Seventy termites in each of three experimental units were acclimated for 2 wk at 25°C and a photoperiod of 10:14 (L:D) h and then subjected to the same decreasing temperatures and change in photoperiod over 6 wk as in the first experiment. After 1 wk at 0°C , the experimental units were returned to 25°C over a 6-wk period by reversing the temperature regime and returning to the original photoperiod for the final 3 wk (Table 1). The units were left undisturbed for the entire 12 wk. At the same time, three other units were kept under control conditions (25°C , 14:10 [L:D] h) for 14 wk.

Percentage Body Water Content and Percentage Survival. At the end of each week during the 6-wk experiment and at the end of the 12-wk experiment, three experimental and three control units were removed and all living termites were carefully extracted, counted, weighed, frozen at -80°C and dried for 3 d at 75°C in a desiccator containing anhydrous calcium chloride. They were weighed daily thereafter until three consecutive weighings were within 0.1 mg of each other to obtain the dry weight. Percentage body water content was calculated using the mean fresh and

Table 2. Comparison of mean percentage survival (\pm SD) of *R. flavipes* workers exposed to constant or decreasing thermophotoperiod for 6 wk

Treatment	Time, wk					
	1	2	3	4	5	6
Decreasing thermophotoperiod	93.8 \pm 2.8	88.9 \pm 2.8*	90.7 \pm 4.8	79.1 \pm 2.0	84.9 \pm 3.4	86.7 \pm 2.3*
Control	88.0 \pm 2.7	82.7 \pm 2.7	83.6 \pm 0.8	78.7 \pm 2.7	79.6 \pm 6.3	69.3 \pm 7.0

*Significant difference between experimental and control values at $P = 0.05$, t -test.

dry weights. Percentage survival was calculated from the number of living termites.

Percentage Fat Content. Percentage fat content of termites in both the 6- and 12-wk experiments was determined according to Layne and Medwith (1997). Dried termites were homogenized in cold (0°C) 2:1 chloroform:methanol (vol:vol) and centrifuged for 18 min. at 4,000 \times g . The supernatant was removed and 0.85% KCl was added in an amount $\frac{1}{4}$ that of the supernatant. Three drops of chloroform:methanol solution were added and the bottom layer of solution was transferred to a preweighed aluminum weighing pan. The solvent was evaporated at \approx 30°C and the pans were reweighed to determine the total weight of fat in each sample. Percentage water and fat content was determined for three samples of 25 workers taken from the laboratory colony and these values served as a baseline for comparison with experimental and control treatments.

Statistical Analyses. All percentage survival and water and fat content data were arcsine transformed. Survival in the control and experimental units was compared and tested for differences with t -tests. Differences in percentage water and fat content by treatment (control, experimental or laboratory colony) or within treatment (by week) for the 6-wk experiment were tested by analysis of variance (ANOVA) and means were separated using Fisher's least significant difference (LSD) test. Controls from the 12-wk study were not used in analyses because of complete mortality in two of the replicates. Therefore, comparisons of percentage water and fat content, using t -tests, were made between experimental and laboratory colony termites only. All analyses were conducted using SAS (SAS Institute 1996).

Results

Subterranean Termite Survival. Overall, percentage survival for the entire 6-wk period was highest for termites exposed to the decreasing thermophotoperiod

than for termites held at constant temperature and photoperiod ($t = 3.5$, $df = 17$, $P = 0.001$) (Table 2). Survival was significantly higher for termites exposed to the thermophotoperiod than their respective controls at 2 and 6 wk ($t = 2.7$, $df = 2$, $P = 0.05$ and $t = 4.5$, $df = 2$, $P = 0.01$, respectively), whereas there were no significant differences in survival between treated and control termites after 1, 3, 4, and 5 wk ($t = 2.6$, $df = 2$, $P = 0.06$; $t = 2.2$, $df = 2$, $P = 0.16$; $t = 0.22$, $df = 2$, $P = 0.84$; and $t = 1.25$, $df = 2$, $P = 0.28$, respectively). In general, survival for both controls and workers exposed to the thermophotoperiod was higher during the first 3 wk than the last 3 wk ($F = 4.99$; $df = 6, 20$; $P = 0.006$; $F = 6.29$; $df = 5, 17$; $P = 0.004$; for control and experimental, respectively). For the 12-wk study, survival in the remaining control was 73.5% and 71.6 \pm 2.04% in the experimental units.

Percentage Water Content. Percentage water content did not significantly differ among control, experimental, or laboratory colony termites over the entire 6-wk period ($F = 2.30$; $df = 2, 53$; $P = 0.11$) (Table 3). The only significant difference was found after week 5 when it was higher in the laboratory colony and control workers than in the workers subjected to the decreasing thermophotoperiod ($F = 11.09$; $df = 2, 8$; $P = 0.01$). There were no significant differences after week 1 ($F = 3.52$; $df = 2, 8$; $P = 0.10$), week 2 ($F = 0.50$; $df = 2, 8$; $P = 0.63$), week 3 ($F = 0.96$; $df = 2, 8$; $P = 0.44$), week 4 ($F = 2.66$; $df = 2, 8$; $P = 0.15$), or week 6 ($F = 0.74$; $df = 2, 8$; $P = 0.51$). Water content did not change significantly over time for both control and experimental workers ($F = 1.49$; $df = 6, 20$; $P = 0.25$; $F = 2.15$; $df = 5, 17$; $P = 0.13$; respectively). But in the laboratory colony, water content was generally higher during the first 3 wk than the final 3 wk ($F = 5.24$; $df = 6, 20$; $P = 0.005$). Water content of workers subjected to a changing thermophotoperiod for 12 wk was 77.4 \pm 0.01%, which was not significantly different ($F = 0.41$, $df = 2$, $P = 0.06$) than the 75.2 \pm 0.01% water content of laboratory colony workers. Percentage water content for workers in the remaining control was 73.5%.

Table 3. Comparison of mean percentage water content (\pm SD) of *R. flavipes* workers exposed to constant or decreasing thermophotoperiod or ambient laboratory conditions for 6 wk

Treatment	Time, wk					
	1	2	3	4	5	6
Decreasing thermophotoperiod	78.8 \pm 0.6	77.5 \pm 0.4	75.4 \pm 3.9	77.1 \pm 0.8	75.0 \pm 0.6*	75.8 \pm 1.4
Laboratory colony	79.6 \pm 1.0	77.9 \pm 0.8	78.0 \pm 1.1	76.5 \pm 0.4	76.5 \pm 0.4	77.0 \pm 0.1
Control	78.0 \pm 0.5	77.8 \pm 0.2	77.6 \pm 1.4	77.9 \pm 0.9	77.4 \pm 0.7	76.2 \pm 1.6

* Significantly lower than laboratory and control termites; ANOVA, LSD test.

Table 4. Comparison of mean percentage fat content (\pm SD) of *R. flavipes* workers exposed to constant or decreasing thermophotoperiod or ambient laboratory conditions for 6 wk

Treatment	Time, wk					
	1	2	3	4	5	6
Decreasing thermophotoperiod	19.0 \pm 1.0a	16.9 \pm 0.4ab	16.0 \pm 2.1a	18.9 \pm 0.6a	21.4 \pm 0.6a	20.2 \pm 4.7a
Laboratory colony	15.5 \pm 2.6a	20.2 \pm 2.7a	19.7 \pm 1.7a	20.9 \pm 1.2a	21.9 \pm 1.2a	25.0 \pm 2.1a
Control	17.9 \pm 1.1a	14.6 \pm 2.3b	16.6 \pm 2.1a	20.5 \pm 2.7a	20.1 \pm 0.4a	22.0 \pm 1.0a

Means within a column followed by the same letter are not significantly different; ANOVA, LSD test.

Percentage Fat Content. Percentage fat content during the entire 6-wk period did not significantly differ among termites exposed to decreasing thermophotoperiods, those kept at constant thermophotoperiod, or termites from the laboratory colony ($F = 2.24$; $df = 2, 53$; $P = 0.12$) (Table 4). The only significant difference occurred at week 2 when it was highest for workers from the laboratory colony ($F = 5.41$; $df = 2, 8$; $P = 0.04$). There were no differences in fat content after week 1 ($F = 3.02$; $df = 2, 8$; $P = 0.12$), week 3 ($F = 2.86$; $df = 2, 8$; $P = 0.13$), week 4 ($F = 1.06$; $df = 2, 8$; $P = 0.40$), week 5 ($F = 3.17$; $df = 2, 8$; $P = 0.11$), or week 6 ($F = 1.91$; $df = 2, 6$; $P = 0.23$). Fat content did not change significantly over time for workers exposed to a decreasing thermophotoperiod ($F = 2.52$; $df = 5, 17$; $P = 0.09$) but there was a general trend for increasing fat content over time for control and laboratory colony termites ($F = 7.43$; $df = 6, 20$; $P = 0.001$ and $F = 5.58$; $df = 6, 20$; $P = 0.004$; respectively). In the 12-wk study, fat content was $28.9 \pm 0.02\%$ for the laboratory colony workers, which was significantly greater ($F = 0.77$, $df = 2$, $P = 0.003$) than the $15.34 \pm 0.03\%$ fat content of termites subjected to changing thermophotoperiods.

Discussion

Partial dehydration can increase an insect's cold hardness (freeze tolerance and resistance) by increasing the concentration of cryoprotectant solutes, such as glycerol, sorbitol, and ethylene glycol, in the hemolymph or the quantity of unfreezable "bound" water in the tissues (Zachariassen 1985, Worland et al. 1998). This results in less water available for freezing and increases the osmolarity of the hemolymph, which lowers the supercooling point (SCP) (the temperature at which ice crystals form). Some insects reduce their water content quite drastically. The arctic collembolan *Onychiurus arcticus* (Tullberg) reduces its body water content from 70 to 40% (Worland et al. 1998) and the beetle *Pytho deplanatus* dropped from 69 to 39% body water content (Ring 1982) as temperatures approached freezing. Frozen insects probably do not lose much water because the frozen body fluids remain in vapor pressure equilibrium with ice and many hibernate in a closed hibernaculum, which reduces contact with ice in the surrounding environment (Zachariassen 1985). Thus, some freeze-tolerant insects do not lose significant amounts of water during cold acclimation. Conversely, insects that hibernate in a supercooled state may become substantially dehy-

drated because water is likely to leave the body fluid and enter external ice crystals in the microhabitat. This is because the vapor pressure in equilibrium with ice is less than the pressure in equilibrium with supercooled water at the same temperature. Therefore, many insect species do not have a reduction in total body water during cold hardening or while in a frozen state (Storey and Storey 1988). Husby (1980) found a low osmotic pressure in the hemolymph of winter-collected *R. flavipes* workers, indicating either few or no cryoprotectants are produced or, as he suggested, a switch to fat metabolism due to the cessation of hindgut protozoan activity resulting in lower levels of sugars in the hemolymph. Fat content decreased slightly in our study during the first 3 wk but this trend did not continue as temperatures dropped below 10°C, perhaps due to a decreased metabolic rate. Fat content also did not differ significantly from the controls or laboratory colony termites, suggesting that lipid reserves are not stored before winter. The general trend for the increase in fat content over time in control and laboratory colony termites and, to a lesser extent, in the experimental termites (though not statistically significant), cannot be readily explained. A similar phenomenon was observed in the 12-wk study with the fat content of laboratory colony workers being almost twice that of the experimental termites. Perhaps this higher fat content is due to continuous activity and feeding, which does not occur under natural conditions as temperatures become colder.

Another strategy used by some insect species to reduce freezing is to void their gut contents (Zachariassen 1985, Duman et al. 1991). This eliminates freezable water and removes particles, such as food, that might serve as initiation sites for ice crystal formation. The relatively stable water content in the experimental termites over time and the lack of significant differences compared with the control and laboratory colony termites suggests that *R. flavipes* does not reduce its body water content or void its gut contents before winter. The latter seems especially unlikely for termites because they would lose their gut fauna and become incapable of obtaining nutrition from ingested cellulose.

We found that survival was higher for *R. flavipes* workers exposed to a decreasing thermophotoperiod than for those kept under constant conditions. Gradually decreasing temperatures with a daily thermoperiod may serve as cues for *R. flavipes* to move deeper into the soil where the temperatures are higher. They may also elicit physiologic responses, such as the pro-

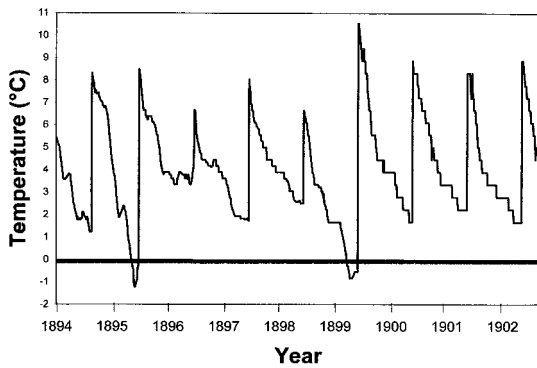


Fig. 1. Daily soil temperatures in Lincoln, NE, recorded at a depth of 91.4 cm during the months of December, January, and February from 1894 to 1902.

duction of cryoprotectants, which enhance cold tolerance. Davis and Kamble (1994) found evidence for cold acclimation in *R. flavipes* when they discovered that workers held at 10°C for 30 d and then subjected to 0°C for 30 d had a lower SCP than termites acclimated for 0, 1, 10, or 20 d. However, this was offset by high mortality so that only a few termites had acclimated. The lower lethal limit (LLL, the temperature at which irreversible knockdown occurs) was higher than the SCP for termites acclimated at 10°C for 0, 1, or 10 d, indicating that the termites died before they froze. However, the opposite occurred when termites were exposed to 10°C for 20 d and the LLL was lowest in termites collected in November, indicating a change from prefreeze mortality in the summer to freeze tolerance in the fall and winter.

Strack and Myles (1997) observed downward movement of *R. flavipes* as temperatures decreased, while Esenther (1969) and Husby (1980) found *R. flavipes* at depths >100 cm during the winter, indicating that *R. flavipes* seeks temperatures that ensure colony survival. In our 12-wk experiment, >70% of the termites survived exposure to temperatures $\leq 5^\circ\text{C}$ for 3 wk, indicating that a large percentage of the colony can tolerate and survive temperatures down to 0°C. However, the higher survival in termites exposed to a decreasing thermophotoperiod also could be due to low temperature delaying mortality that may have occurred sooner at warmer temperatures. Experiments are also needed to determine whether photoperiod affects *R. flavipes* because it has minimal exposure to light underground.

Soil temperatures recorded in Lincoln, NE, during the months of December, January, and February at a depth of 91.4 cm from the years 1894–1902 (Swezey 1903) reveal that temperatures rarely dropped below 0°C (Fig. 1). Based on these temperatures, and assuming that soil temperature profiles have changed very little over the past 100 yr, it seems unlikely that *R. flavipes* colonies are ever exposed to sub-zero temperatures during winter if they move >100 cm underground. The lowest temperature of -1.22°C recorded by Swezey (1903) is higher than the LLL of

-3.0 and -2.9°C for *R. flavipes* workers and soldiers, respectively (Sponsler and Appel 1991). Davis and Kamble (1994) reported higher LLLs of -5.56 to -6.53°C for laboratory-reared *R. flavipes* workers acclimated at 10°C from 0 to 30 d, and -4.93 to -6.80°C in field-collected *R. flavipes* workers. However, the LLL is determined in less than ≈ 2 h, whereas termites under natural conditions experience a much slower cooling rate over a longer time so that the actual LLL under natural conditions might be considerably different. The soil temperatures at 91.4 cm were also much higher than the SCPs of -5.38 to -10.18°C in laboratory-reared or field-collected *R. flavipes* workers (Davis and Kamble 1994), indicating that the risk of freezing during the winter is low. Strack and Myles (1997) observed very little activity of *R. flavipes* workers at 5°C and none at 0°C, and Sponsler and Appel (1991) determined a critical thermal minimum (the temperature at which reversible knockdown occurs) of 13.3 and 12.1°C for *R. flavipes* workers and soldiers, respectively. However, *R. flavipes* in cold torpor can recover quickly when warmed. Beard (1974) observed *R. flavipes* workers resuming activity soon after being removed from a small log that had been outdoors where air temperatures had been as low as -17°C .

Our results indicate that *R. flavipes* can survive prolonged exposure to cold temperatures thus enabling them to survive the winter in the colder regions of its range. The evidence suggests that *R. flavipes* passes the winter in an inactive state at temperatures above freezing. Measurements of the actual soil temperatures during the winter at depths ≥ 100 cm are needed as well as the identification of any cryoprotectants in the hemolymph that enhance cold tolerance.

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