Supercooling Differences in the Eastern Subterranean Termite (Isoptera: Rhinotermitidae)[1]

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Abstract Supercooling points were determined for untreated field-collected and untreated laboratory-maintained *Reticulitermes flavipes* (Kollar) workers and soldiers. Workers treated with antibiotics or had their hindgut-protozoa removed by exposing them to oxygen under pressure to determine the effects of absence of the hindgut fauna on supercooling. Supercooling points were compared between live and freshly-killed workers to determine whether supercooling in this species might be simply due to the biochemical properties of body fluids. Laboratory-maintained workers were also subjected to desiccation, starvation, or atmospheric pressure to determine their effects on supercooling. Supercooling points were lowest for laboratory workers treated with antibiotics and those that fed on brown paper-toweling for 7 d. Untreated field-collected workers had significantly higher supercooling points than untreated laboratory-maintained workers (−6.06 ± 0.79°C vs −9.29 ± 2.38°C, $P < 0.0001$). Both untreated field-collected and laboratory soldiers had significantly lower supercooling points than their respective workers (−7.39 ± 2.01°C vs −6.06 ± 0.79°C, $P < 0.0001$; and −11.60 ± 2.53°C vs −9.29 ± 2.38°C, $P < 0.0001$, respectively). There was no significant association between termite body mass and supercooling points for both laboratory and field termites ($P = 0.0523$ and $P = 0.6242$) or water content of laboratory termites and supercooling points ($P = 0.1425$). Defaunated workers had significantly lower supercooling points (−10.34 ± 2.38°C) than normally faunated workers (−9.48 ± 1.85°C)($P = 0.0095$) suggesting that the symbiotic fauna may have higher supercooling points and act as ice nucleators in the termite hindgut. Starved and desiccated workers had significantly lower supercooling points (−10.38 ± 2.70°C and −10.39 ± 2.38°C, respectively) than their corresponding control groups (−9.87 ± 2.11°C and −9.89 ± 1.94°C; $P = 0.0454$; $P = 0.0234$, respectively) and untreated workers (−9.29 ± 2.38°C; $P = 0.0021$; $P = 0.0011$) suggesting that some forms of physical stress might lower the supercooling point.

Key Words *Reticulitermes flavipes*, supercooling, overwintering, hindgut, bacteria, protozoa

Insects that overwinter in climates with freezing temperatures survive using one of two strategies: freeze tolerance or freeze avoidance (Zachariassen 1985, Storey and Storey 1988, Block 1990). In freeze-tolerant insects, ice crystals form at higher subfreezing temperatures and their growth is controlled to reduce intra- and extra-cellular damage caused by rampant ice crystal formation (Storey and Storey 1988). Ice formation can be controlled by cryoprotectants, such as glycerol (Storey 1990), or ther-

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mal hysteresis proteins (Storey and Storey 1988, Block 1990) and can be induced by ice-nucleating agents (INAs) (Duman et al. 1991c) such as food particles (Block 1990, Duman et al. 1991b, Storey et al. 1993, Zachariassen 1992, Klok and Chown 1997) or bacteria in the gut (Duman et al. 1991a, Sinclair 1997). Freeze-avoiding insects can reduce ice formation by producing antifreeze compounds such as glycerol (Block 1990, Storey 1990, Duman et al. 1991b), ethylene glycol, sorbitol and mannitol (Zachariassen 1985, Duman et al. 1991b), trehalose (Storey 1990, Storey et al. 1993) or antifreeze proteins (Block 1990, Duman et al. 1991b). These compounds lower the supercooling point (SCP), the temperature at which a liquid spontaneously freezes (Lee 1991), so that freezing occurs at much lower temperatures than in freeze-tolerant insects. Removal or inactivation of ice-nucleating proteins and INAs (Zachariassen 1985) or voiding of gut contents (Zachariassen 1985 and 1992, Duman et al. 1991b) can also enhance supercooling.

Insect overwintering has been extensively researched (Lee 1991), but relatively few studies have involved termites. The eastern subterranean termite, *Reticulitermes flavipes* (Kollar), is found in areas within its endemic range, such as Wisconsin (Esenther 1969) and Ontario, Canada (Husby 1980, Strack and Myles 1997), where sub-zero temperatures occur during the winter. Thus, it must have both physiological and behavioral adaptations for surviving long-term exposure to low temperatures. Esenther (1969) and Husby (1980) reported *R. flavipes* retreating deep underground or into large pieces of wood during the winter, most likely to avoid freeze mortality.

One characteristic of lower termites (species other than those in the family Termitidae), such as *R. flavipes,* is the presence of symbiotic protozoa and bacteria in the hindgut that contribute to the host's energy requirements (Bignell 2000). Is it possible these organisms could initiate ice crystal formation in the hindgut if they froze at a higher temperature than their hosts? Klok and Chown (1997) found that starved *Pringleophaga marioni* Viette caterpillars, a species with a well-developed gut flora, had lower SCPs than fed caterpillars leading them to conclude that gut contents function as INAs.

In the present study, we investigated how several factors, including the presence or absence of the hindgut fauna, water content, body mass, starvation, and caste might affect supercooling in *R. flavipes.* We also compared the SCPs of field-collected and laboratory-maintained *R. flavipes* soldiers and workers.

Materials and Methods

Termites. Two different groups of termites were used in this study: laboratory and field termites. Termites from the first group were collected in Lincoln, NE, and maintained in the laboratory at approximately 23°C in a 57-L glass container with moist soil and sand. A glass plate covering the container reduced moisture loss; ash blocks and pine stakes on the soil surface served as a food source. The termites had been kept under these conditions for at least 9 months prior to beginning the experiments. Field termites were collected from five different sites in Lincoln on 24 June, 28 July, 20 August, 15 September, and 27 September of 1999 from in-ground traps consisting of damp, corrugated cardboard rolls inside covered, 3.8-L plastic buckets with the bottoms removed. Field termites were transferred to covered plastic containers (30 × 15 × 10 cm) containing damp paper toweling and taken to the laboratory where SCPs were usually determined within 3 d after they were collected.

Supercooling point determination. Nine thermocouples (Type T, Kapton, Cole-

Parmer Instrument Company, Vernon Hills, IL) were threaded through nine hollow glass rods (≈6 cm long) inserted through rubber stoppers. Nine termites were individually weighed to the nearest 0.1 mg and attached to the thermocouples by coating the tips with a small amount of petroleum jelly and pressing them gently to the termites' dorsum. After attaching the termites, the stoppers were fitted onto nine 30-ml glass tubes in a test tube rack so that the termites were suspended inside and approximately midway down the center of each tube. The rack was placed in a water bath (model RTE-210, Neslab, Portsmouth, NH) filled with 12-L of a 1:1 (vol:vol) mixture of distilled water and ethylene glycol. The temperature was lowered at a rate of 0.2 to 0.5°C/min; termite temperatures were recorded and stored every 4 s with a scanning digital thermometer (bench model 692-0000, Barnant Company, Barrington, IL). The SCP was identified as the lowest temperature recorded before the exotherm produced by the latent heat of crystallization occurring in the termite. The exotherm was clearly visible on the scanning thermometer program's on-screen graphics display as a spike in temperature increase. None of the termites survived the SCP determination procedure.

Untreated termites. Laboratory termite workers were taken directly from the glass container and their supercooling points were immediately determined. These termites did not undergo any kind of treatment nor were they manipulated in any way.

Antibiotic treatment. Several antibiotics have proved effective in killing bacteria in the termite hindgut (Eutick et al. 1978, Mauldin and Rich 1980). Based on these studies and preliminary testing, we found that a 0.5% tetracycline/kanamycin (1:1) solution was the most effective in removing the hindgut bacteria and was not acutely toxic to *R. flavipes*. Thirteen drops (approximately 4 to 5 ml) of this solution, were applied to filter paper disks (Whatman No. 1, 90 mm diam.). After air-drying, the disks were placed in plastic Petri dishes (100 × 15 mm), moistened with 3 drops of distilled water. Twenty-five *R. flavipes* workers were added to each dish. The dishes were sealed with Parafilm™ (American National Can, Chicago, IL), wrapped in foil, kept at ambient room temperature (≈23°C) and water added as needed to maintain adequate humidity. After 7 d, the termites were removed for SCP determination. After several trials using filter paper, the antibiotic treatments were repeated using paper toweling (Georgia Pacific Inc., Atlanta, GA) to see if differences in paper type might affect the results (Cook and Gold (2000) found the source of cellulose affected hindgut flagellate communities in *R. virginicus*). Controls for antibiotic treatments on filter paper and paper toweling consisted of approximately 4 to 5 ml of distilled water applied to the paper and the termites held under the same conditions as the treatments.

Defaunation. A technique similar to that of Holmes (1970) was used to defaunate termites. The defaunation chamber was a 0.95-L pressure cooker with a pressure gauge fitted to the lid. Oxygen from a cylinder was introduced into the cooker through Tygon™ tubing connected to the cylinder regulator and the steam vent on the lid. An open 237-ml canning jar, containing several damp paper towel disks and approximately 20 to 25 termites, was placed inside the cooker. A stiff paper baffle was placed in front of the jar to deflect the flow of oxygen coming in through the vent. The cooker was loosely covered and flushed with oxygen for approximately 2 min. After flushing, the lid was locked, and the pressure was gradually increased to and maintained at 77.55 mm Hg for 6 h (preliminary tests revealed this was the minimum time needed for complete defaunation). After treatment was completed, the pressure in the cooker was released gradually to avoid rapid decompression. The termites were removed, weighed, and their SCPs determined within 24 h of defaunation. Controls consisted of

termites held concurrently for 6 h under ambient room conditions in an open 237-ml canning jar provisioned with damp filter paper disks.

Air pressure treatment. Termites were given the same pressure treatment with air instead of oxygen to see if increased atmospheric pressure had an affect on the SCP. This treatment does not cause defaunation. The corresponding controls were termites kept under the same conditions as the controls for the defaunation treatment.

Ethyl acetate treatment. To determine if supercooling might simply be due to the biochemical properties of body fluids, termites were maintained for 10 min in an insect kill jar charged with ethyl acetate and immediately weighed. The SCPs of these dead termites were recorded while SCPs of live laboratory termites were determined and served as controls.

Starvation. Twenty to 25 termites were placed in an empty, 100-mm plastic Petri dish. The bottom and lid were roughened with sandpaper, the former to provide traction for the termites and the latter to improve adherence of water droplets added to maintain a high relative humidity inside the dish. Twenty to 25 termites placed in a dish provisioned with filter paper served as a control. The dishes were sealed with Parafilm™, wrapped in foil, and kept at ambient room temperature (≈23°C) for 7 d. - At the end of this period, SCPs were determined for both starved and control termites.

Water content and desiccation. Because we observed a wide variation in the SCPs of laboratory termites, the percentage water content was determined for 18 laboratory colony workers and their SCPs were determined to see if there was a relationship between water content and supercooling. After SCPs were recorded, the termites were dried on filter paper and the percent water content was calculated according to Cabrera and Kamble (2001). Additionally, 36 more workers were weighed, placed individually in small, plastic Petri dishes (60 × 15 mm) and held in desiccators containing Drie-rite® (W.A. Hammond Drierite Company Ltd., Xenia, OH) for 6 to 6.5 h. At the end of this period, seven individuals were dead or moribund. The remaining 29 workers were reweighed, and their SCPs were determined as described previously. Thirty-six workers held for 6 to 6.5 h in petri dishes provisioned with damp paper toweling served as controls.

Field termites. Supercooling points were determined for untreated termites brought directly in from the field, usually within two days after they were collected. Supercooling points were also determined for field termites that were defaunated, treated with air pressure, or starved, and their corresponding controls. Percentage water content also was determined for 24 workers. Because of the limited number of termites obtained during each collection trip, no field termites were treated with antibiotics.

Soldiers. Supercooling points were determined for untreated soldiers whenever they became available (soldiers are present in lower numbers than workers). A few were experimentally treated, but because there were not very many, their SCPs were not included in the statistical analyses.

Statistical analyses. Differences in the SCP between untreated workers and experimentally-treated laboratory workers for both laboratory and field workers were analyzed using the Kruskal-Wallis test and Wilcoxon rank-sum tests (SAS 2001, PROC NPAR1WAY). Differences between treated workers and their respective controls and between untreated workers and soldiers, for both laboratory and field termites, were compared using Wilcoxon rank-sum tests. Non-parametric tests were

used because the data were not normally distributed. Kendall rank correlation coefficient analyses (Kendall's Tau b) (PROC CORR) were conducted to measure the associations between mass and the SCP of untreated field and laboratory workers and percentage water loss and the SCP of laboratory workers.

Results

Laboratory colony. Treatments applied to workers had a significant effect on SCP (df = 7; X^2 = 96.28; $P < 0.0001$). Workers feeding on filter paper or paper toweling treated with antibiotics had the lowest SCPs while untreated workers and those held under 77.55 mm Hg air pressure for 6 h had the highest SCPs (Table 1). Antibiotic-treated (on both filter paper and paper toweling), defaunated, starved, and desiccated workers had significantly lower SCPs than untreated workers ($P < 0.0001$, $P < 0.0001$, $P = 0.0012$, $P = 0.0021$, and $P = 0.0011$, respectively). The SCPs of untreated workers and those exposed to increased air pressure or freshly killed after exposure to ethyl acetate fumes were not significantly different ($P = 0.1122$; $P = 0.0655$, respectively). Workers which had been defaunated, desiccated, starved, or had fed on filter paper treated with antibiotics had significantly lower SCPs than their corresponding controls ($P = 0.0095$; $P = 0.0454$; $P = 0.0234$, $P < 0.0001$, respectively). The SCPs of workers that fed on paper toweling treated with antibiotics did not differ significantly from those of workers fed on untreated paper toweling ($P = 0.3442$), but those feeding on antibiotic-treated filter paper had significantly lower SCPs than those feeding on untreated filter paper ($P < 0.0001$). There were no significant differences between the SCPs of workers killed by ethyl acetate or exposed to elevated air pressure and their respective controls ($P = 0.0549$ and $P = 0.1695$, respectively). Soldiers had significantly lower SCPs than untreated workers (−11.60 ± 2.53°C vs −9.29 ± 2.38°C., $P < 0.0001$) (Table 1).

Field-collected termites. Treatment had some significant effects on SCPs (df = 3, X^2 = 93.3, $P < 0.0001$). Ethyl acetate-killed workers had the lowest and starved workers had the highest SCPs (Table 1). Defaunated worker SCPs were slightly, though significantly lower than the SCPs of untreated workers ($P = 0.0475$). Soldiers had significantly lower SCPs than untreated workers ($P < 0.0001$). Defaunated, acetate-killed, and starved worker SCPs were significantly lower than their corresponding controls ($P = 0.0444$; $P < 0.0001$; $P < 0.0001$, respectively).

Laboratory vs. field termites. Both untreated workers and soldiers from the laboratory colony had significantly lower SCPs (−9.29 ± 2.38°C and −11.60 ± 2.53°C, respectively) than untreated workers and soldiers collected from the field (−6.06 ± 0.79°C and −7.39 ± 2.01°C, respectively) ($P < 0.0001$ and $P < 0.0001$) (Table 1).

Effect of mass and water loss on supercooling. Live mass and SCP were slightly associated for laboratory workers (N = 111, $P = 0.0523$, $\tau = 0.1301$) (Fig. 1) but there was no significant association for field workers (N = 130, $P = 0.6242$, $\tau = -0.0306$) (Fig. 2). There was no significant association between percentage water loss and SCP (N = 35, $P = 0.1425$, $\tau = 0.1765$) (Fig. 3).

Discussion

Factors affecting supercooling in other insects include the presence of ice-nucleating bacteria (Strong-Gunderson et al. 1990), gut contents (Shimada 1989, Klok and Chown 1997, Hart and Bale 1998, Worland et al. 1998), field versus labo-

Table 1. Mean supercooling points (±SD) of laboratory-maintained and field-collected R. flavipes workers and soldiers subjected to various treatments

Treatment	Supercooling point (°C)							
	Laboratory colony				Field-collected			
	n	Treated	n	Control	n	Treated	n	Control
None	111	−9.29 ± 2.38	—	NA	130	−6.06 ± 0.79	—	NA
Air pressure	70	−9.09 ± 2.94	65	9.54 ± 31.0	—	—	—	—
Antibiotics on filter paper	35	−13.27 ± 1.31	34	−11.91 ± 1.96	—	—	—	—
Antibiotics on paper toweling	26	−12.56 ± 1.74	27	−12.69 ± 1.57	—	—	—	—
Desiccation	29	−10.39 ± 2.38	36	−9.89 ± 1.94	—	—	—	—
Ethyl acetate	58	−9.82 ± 2.46	67	−9.20 ± 2.57	50	−7.36 ± 14.0	44	−6.21 ± 0.48
Defaunation	68	−10.34 ± 2.38	66	−9.48 ± 1.85	27	−6.20 ± 0.57	25	−5.92 ± 0.39
Starvation	70	−10.38 ± 2.70	68	−9.87 ± 2.11	45	−7.08 ± 1.44	35	−6.06 ± 0.56
Soldier	27	−11.60 ± 2.53	—	NA	16	−7.39 ± 2.01	—	NA

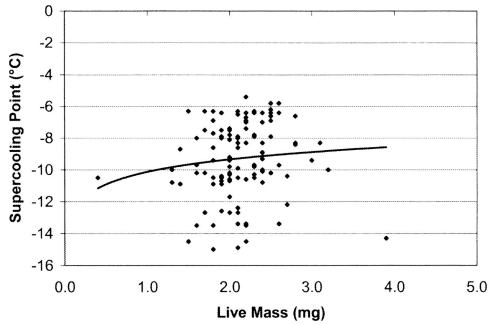

Fig. 1. Plot of live laboratory maintained *R. flavipes* worker masses versus their corresponding supercooling points showing a very slight association (Kendall's rank correlation coefficient, N = 111, $P = 0.0523$, $\tau = 0.1301$).

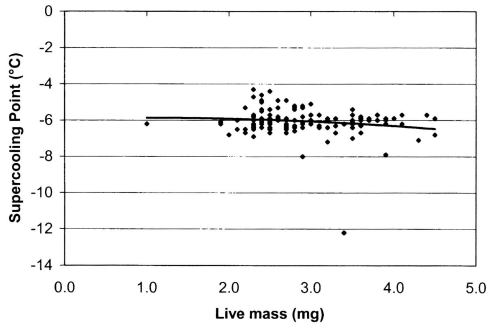

Fig. 2. Plot of live field-collected *R. flavipes* worker masses versus their corresponding supercooling points showing no significant association (Kendall's rank correlation coefficient; N = 130, $P = 0.6242$, $\tau = 0.0306$).

ratory origin, size, and life stage (Kim and Kim 1997), acclimation (Sinclair 1997) and time of year (Ramlov et al. 1992, Layne and Medwith 1997, Sinclair 1997). However, the effects of these factors appear to be species specific. Moreover, differences in individual factors can affect the SCP. For example, Gillyboeuf et al. (1994) suggested that differences in the SCP between two populations of pink maize stalk borer, *Sesamia nonagrioides* Lef, were due to the amounts of INAs in the two corn cultivars the borers fed on. Acclimation to low temperature has resulted in both increased (Tursman et al. 1994, Gehrken and Southon 1996, Sinclair 1997) and decreased (Ramlov et al. 1992, Worland et al. 1998) SCPs while others found no effect (Miller 1978, Gillyboeuf et al. 1994, Kim and Kim 1997). Davis and Kamble (1994) observed lower SCPs in *R. flavipes* workers acclimated at 10°C for 30 d and then exposed to 0°C for 30 d than in workers acclimated for shorter periods. However, they also suffered high mortality suggesting that enhanced cold tolerance occurred in only a few individuals. In contrast, Cabrera and Kamble (2001) found 87% survival of *R. flavipes* workers

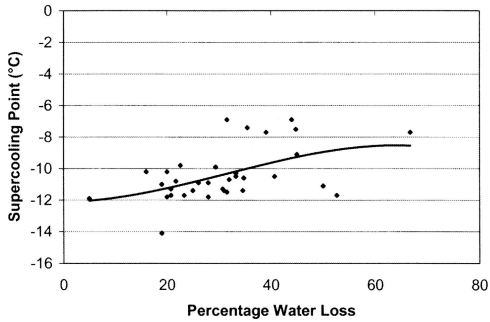

Fig. 3. Plot of percentage water loss of laboratory maintained *R. flavipes* workers and their corresponding supercooling points (Kendall's rank correlation coefficient; N = 35, $P = 0.1425$, $\tau = 0.1765$).

held at temperatures gradually decreasing from 25° to 0°C over 6 wks, including 1 wk at 0°C. Beard (1974) observed that *R. flavipes* inhabiting logs outdoors where ambient air temperatures were as low as −17°C, resumed activity after being brought indoors and warmed to indoor temperatures. Thus, it appears *R. flavipes* can survive long-term exposure to low temperatures.

The underlying mechanisms responsible for the large differences in supercooling we observed between laboratory and field collected termites are unknown. Environment may be a factor because the two populations came from completely different habitats. The laboratory colony had been kept at ambient room conditions at ≈23°C for at least 9 months while the field termites were probably exposed to greater variations in temperature as they foraged under different soil conditions. Davis and Kamble (1994) reported different SCPs in *R. flavipes* workers collected from May through November although the mean SCP was actually higher in workers collected later in the year when average daily temperatures were decreasing. However, they did observe a decrease in lower lethal temperatures (LLLs) in termites collected in November.

The lower SCPs of soldier versus those of workers may not be surprising, given the differences in morphology and physiology between these two castes. Sponsler and Appel (1991) found the critical thermal minimum of *R. flavipes* soldiers to be 1.2°C lower than that of workers. The greater muscle mass and the more heavily sclerotized head capsule of soldiers may be a contributing factor. Another possible explanation may be differences in hindgut contents. Soldiers, with their specialized mandibles, are unable to feed on wood and instead are fed by the workers via trophallaxis. Thus, they may have fewer solids and lower relative numbers of protozoa in their hindgut to act as INAs.

The lower SCPs with a wider variation seen in laboratory termites compared with the higher SCPs with lower variation in field termites could be due to differences in cryoprotectant synthesis or thermotolerance. A lowering of the SCP sometimes, though not always, corresponds with higher cryoprotectant levels in the hemolymph (Duman 1979, Gehrken and Southon 1996). Husby (1980) concluded that *R. flavipes* workers collected during the winter lacked cryoprotectants based on the low hemo-

lymph osmolalities he measured. Qualitative identification of cryoprotectants and ice nucleators in *R. flavipes* is needed to gain a complete understanding of supercooling in this species and the underlying factors producing the large difference in supercooling observed between the laboratory and field colonies.

Defaunation significantly lowered the SCP in laboratory workers compared with their corresponding controls, untreated workers, and workers held at 77.55 mm Hg air pressure suggesting hindgut protozoa affect supercooling in *R. flavipes.* The lower SCPs of starved workers could also be due to a decrease in protozoa numbers (although we did not examine gut contents of starved workers). Perhaps the symbiotic protozoa have a higher SCP than the termites. Freezing protozoa could act as INAs in the hindgut, thus causing the termites to spontaneously freeze at a temperature that is higher than their natural SCP. Defaunated termites do not have the protozoa to act as ice nucleators so they freeze at the SCP of their body fluids. The SCP of the body fluids in field termites may be greater than or similar to the SCPs of the protozoa which may explain the similarity in the observed SCPs of defaunated and untreated field workers.

Kim and Kim (1997) attributed a higher SCP in field-collected beet armyworm larvae, compared with laboratory-reared larvae, to the presence and ingestion of ice-nucleating bacteria in the field. In our study, antibiotic-treated *R. flavipes* workers had the lowest SCPs of all indicating the hindgut bacteria may function as INAs. The mean SCPs of workers feeding on filter paper or paper toweling treated with antibiotics was, respectively, 4 and 3.3°C lower than that of untreated workers. Interestingly, the mean SCPs of workers feeding on untreated filter paper or paper toweling were also very low (−10.81 and −11.91, respectively). These two different materials may not provide adequate nutrition for *R. flavipes* and their hindgut fauna. Cook and Gold (2000) found that different cellulose sources, including filter paper, had significant effects on relative abundances of flagellate species in *R. virginicus.* The low SCPs in the termites feeding on the two types of paper in our study could be a result of decreased numbers of protozoa and/or bacteria in the hindgut. Unfortunately, time constraints and availability of termites did not allow us to treat field workers with antibiotics nor did we treat laboratory workers with both oxygen and antibiotics to make workers devoid of both protozoa and bacteria. Nonetheless, our results suggest that both bacteria and protozoa have ice-nucleating activity in the termite hindgut. However, although emptying of gut contents is one strategy used by some overwintering insects to avoid freezing, it is unlikely termites would do the same because the gut symbionts are vital to their energy requirements. Spontaneous ice crystal formation in *R. flavipes* hindguts in the winter may be less likely because termite activity decreases and eventually stops as soil temperatures drop to 0°C (Strack and Myles 1997). This decrease and stoppage of feeding may result in fewer food particles in the hindgut.

The wood species that subterranean termites feed upon can have a significant affect on hindgut protozoa species (Mannesmann 1974, Carter et al. 1981, Cook and Gold 2000). Perhaps the food source the termites are feeding on in the field termites is affecting their hindgut fauna and consequently the SCP. Comparisons of absolute protozoan numbers and species assemblages in field-collected and laboratory termites could reveal differences in protozoan populations in termites living under natural and laboratory conditions which may account for the marked differences in supercooling.

Neither water content nor termite weight seemed to affect supercooling. These

factors may be more important in larger insects. However, desiccated workers did have lower SCPs than untreated workers. This loss of water somehow affected supercooling perhaps by altering the concentration of particular compounds in the hemolymph. According to Zachariassen (1985), a reduction in body water content increases the concentration of cryoprotectants leading to changes in hemolymph osmolality and the SCP.

There was no significant difference in SCP between live and dead laboratory workers although there was a difference of over 0.5° and dead field workers had lower SCPs than live field workers. The workers may have defecated some fluid upon exposure to ethyl acetate thus reducing the number of INAs in the hindgut. Another possibility is some water could have been lost through open spiracles of the dying termites.

One crucial piece of information needed for understanding termite overwintering and their ability to survive long-term exposure to cold temperatures is the temperature range of the microhabitats where *R. flavipes* colonies overwinter. This would place all known information on *R. flavipes* cold tolerance in a meaningful context. However, because *R. flavipes* is believed to have a diffuse nest system (Strack and Myles 1997) rather than a centralized nest area as do many other subterranean termite species (Weesner 1970) and because locating subterranean termites in the ground is difficult and painstaking work, obtaining exact temperatures would be a difficult task. Measuring the soil temperature at depths at which termites are likely to be found, however, may be sufficient. Mail (1930) stated that daily temperature fluctuations are nonexistent below a certain soil depth; thus, it is likely that termites deep in the ground during the winter are at a near constant temperature. Both Esenther (1969) and Husby (1980) found *R. flavipes* at soil depths of $\geq$1 m in the winter. The lowest temperature recorded by Esenther (1969) in Wisconsin at a soil depth of 122 cm was −1.1°C. Soil temperature data collected in Lincoln, NE, during the months of December, January and February from 1894 to 1902 revealed a mean soil temperature of 3.9°C, at a depth of 91.4 cm, with a minimum and maximum of −1.2 and 10.6°C, respectively (Swezey 1903). These temperatures are considerably higher than the SCPs of *R. flavipes* reported by Davis and Kamble (1994) ($\approx$6.0°C), those of the field termites in this study (−6.1°C), and the lower lethal limits (the temperature at which irreversible knockdown occurs) of −3.0 and −2.9°C for workers and soldiers, respectively, reported for *R. flavipes* by Sponsler and Appel (1991). Thus it is very likely that successfully overwintering *R. flavipes* colonies are seldom exposed to temperatures below 0°C. This may explain how colonies survive the winter in the mid- to upper latitudes of the native range of *R. flavipes*. Thus, considerable winter mortality occurs only when colonies overwinter in sites where temperatures drop down to and below the lower lethal temperature. Termites may also overwinter in the soil adjacent to human dwellings that are heated artificially, thus providing temperatures that are not lethal to termites. Gillyboeuf et al. (1994) concluded that the overwintering microclimate is probably more important than freeze tolerance capacity for the survival of pink maize stalk borer and this probably also holds true for *R. flavipes*.

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