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Seasonal Variation of Critical Thermal Limits and Temperature Tolerance in Formosan and Eastern Subterranean Termites (Isoptera: Rhinotermitidae)

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ABSTRACT Critical temperatures and the upper lethal limit (ULL) of field collected workers of Formosan subterranean, *Coptotermes formosanus* Shiraki, and eastern subterranean termites, *Reticulitermes flavipes* (Kollar), were measured monthly from September 2001 to August 2002. Monthly mean critical thermal maxima (CTMa), ULL, and critical thermal minimum (CTMin) fluctuated significantly throughout the year. Seasonal variations of CTMax and ULL of both species were slightly, but significantly, statistically correlated with seasonal variation of habitat soil temperatures at 15-cm depth. Regression analyses revealed a significant linear relationship between CTMin and habitat temperatures for both termites as well. The two termite species responded positively to temperature changes in a similar manner. The values of all three parameters were significantly greater for *C. formosanus* (CTMax: 44.8–45.9°C, ULL: 49.1–50.4°C, CTMin: 7.2–9.0°C) than *R. flavipes* (CTMax: 43.5–44.9°C, ULL: 46.9–48.3°C, CTMin: 1.0–4.9°C). *R. flavipes* had a wider range between the upper and lower critical temperatures than *C. formosanus*. The three thermal tolerance parameters were not correlated with worker body mass. The significance of the critical and lethal temperatures and comparisons with previous studies are discussed.

KEY WORDS Coptotermes formosanus, *Reticulitermes flavipes*, thermal tolerances, seasonal variation, habitat temperature

TEMPERATURE IS ONE OF THE most important environmental factors that affect termite geographical distributions and local occurrences (Kofoid 1934). Termite survival in high and low temperatures is subject to certain physiological limits. Various parameters have been used to measure temperature limits of organisms. The critical thermal maximum (CTMax) and the critical thermal minimum (CTMin), as well as the upper lethal limit (ULL) and lower lethal limit (LLL), have been used to determine the physiological temperature tolerances of insects and other animals. The critical temperatures are regarded as defining the ecological or behavioral temperature tolerance limits and are consequently of greater value in ecological studies (Mitchell et al. 1993) than the lethal temperatures, which are, for example, valuable in making recommendations on practical modified temperature treatments for control of insects (Scheffrahn et al. 1997).

It is widely believed that the Formosan subterranean termite, *Coptotermes formosanus* Shiraki, was introduced into the United States ≈50 yr ago in wood materials shipped from Asia after World War II (La Fage 1985). Although thought to be native to mainland China and its neighbors in subtropical regions (Kistner 1985), it has rapidly spread into 11 southeastern states in the United States, with northward extensions into the warmer parts of temperate areas, and its spread has shown no sign of slowing (Woodson et al. 2001, Su 2003). Because of its competitive advantages of aggression (Su and Scheffrahn 1988a, Hu and Zhu 2003), large colony size, and voracity (Su and Scheffrahn 1988b), *C. formosanus* is becoming better known as a serious pest in the United States. The destructive activity and rapid spread of *C. formosanus* have caused public concern about its potential to expand its range even farther north and inland to areas previously thought to be uninhabitable (Woodson et al. 2001). In China, it was reported that, in nature, *C. formosanus* could survive in coastal areas with mean January temperatures of ≥4°C and mean minima of ≥ −5°C (Li 1991). Strikingly, it is now established in Cullman county, inland northern Alabama, where the mean winter temperature is ≥2°C, mean minimum temperature is ≥ −4°C, and the low temperature extreme is −15°C (unpublished data).

There is only a single study that explored the critical thermal limits (CTs) of *C. formosanus* (Sponsler and Appel 1991), and two studies that measured its ULLs (Sponsler and Appel 1991, Woodrow and Grace 1998). There are a few studies on the overwintering biology of the eastern subterranean termite, *Reticulitermes flavipes* (Kollar) (Davis and Kamble 1994, Cabrera

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and Kamble 2001). *R. flavipes* is another damaging termite that is broadly distributed across the southeastern states, where it coexists with *C. formosanus*, to temperate regions with severe winters as far north as Kincardine, Ontario (Strack and Myles 1997), and Janesville, WI (Esenther 1969). However, these studies do not address the effect of seasonal temperature variation on termite temperature tolerances. Physiological capabilities for temperature acclimatization that might allow termites to survive cold winters remain unknown. Insect survival ability at high or low temperature is related to seasonal habitat temperatures. Positive correlations have been found between the CTMax and the habitat temperature of cockroaches (Tsuji and Mizuno 1973, Appel et al. 1983), and the red imported fire ant, *Solenopsis invicta* Buren (Cokendolpher and Phillips 1990).

This study was undertaken to better understand the physiological limits of *C. formosanus* and *R. flavipes* and to elucidate the influences of seasonal habitat temperature fluctuations on CTs and ULL. Therefore, CTs and ULL of field-collected termites were measured at monthly intervals during a 1-yr period. The objectives were four-fold: first, to assess the temperature tolerance limits (CTMax, CTMin, and ULL) of the two subterranean termite species; second, to determine if CTMax, CTMin, and ULL of each termite species fluctuate with yearly seasonal temperature variation; third, if these species do respond to temperature changes, to determine if there is any relationship between the CTs or ULL and seasonal soil habitat temperatures at 15-cm depth; and fourth, to examine if the temperature tolerance limits are associated with termite body mass.

Our intent was to provide a more comprehensive understanding of the potential capability of *C. formosanus* and *R. flavipes* to survive extreme temperatures. We measured termite worker thermotolerance to seasonal changes of habitat temperature at a 15-cm depth, because the worker caste is accountable for colony introduction into new areas in infested wood products (Forschler and Gauntt 2000, Howell et al. 2001). Hence worker physiological thermal tolerances may provide insight into the potential for spread or successful introduction of *C. formosanus* into areas outside its current geographic range in the United States.

Materials and Methods

Termite Collection and Preparation. Termites were collected from field colonies in Auburn, Lee Co., AL. Termite foraging groups were associated by colony using the triple mark-release-capture technique (Su et al. 1993) and were identified to species using taxonomic keys for soldiers (Scheffrahn and Su 1994). Underground open-bottom plastic bucket traps (18 cm high by 13 cm diameter), two for each colony, were designated for termite collections. Traps were baited with bundles of spruce wood (*Pinus pungens* Engelm) tied with rubber bands. Voucher specimens of each colony are stored in 100% ethyl alcohol in the Insect Collection of the Department of Entomology and Plant Pathology, Auburn University, Auburn, AL. Termites were collected in the middle of each month from September 2001 to August 2002.

We started with five *R. flavipes* colonies (colonies 1, 2, 3, 4, and 5) and four *C. formosanus* colonies (colonies I, II, III, and IV) from designated field traps and attempted to use all the colonies for the year-long study. All the colonies were in urban residential areas. However, during winter months, some termite colonies abandoned the trap sites for various extended periods, some revisited, and some never returned. During the spring of 2003, pest control companies treated several structures close to our trap sites without our knowledge and killed colonies II and E. As a result, we did not obtain data from all the colonies each month, although we checked the traps every month and made collections if there were termites.

At each collection, the rubber bands were untied, and the wood bundle was gently opened one piece at a time to collect foraging workers. Termites were tapped or brushed gently into a plastic container lined with a moist paper towel and transported immediately to the laboratory for testing. After each termite collection, the entire wood bundle was retied, placed back in the trap, and covered with a plastic lid. If more than one-half of the wood in a trap was consumed, it was replaced with new moistened wood.

To investigate the relationship between ground temperature and the CTs and ULLs, a temperature measurement was taken, using a water-resistant stainless steel digital thermometer (Professional Equipment, Hauppauge, NY), inserted to a depth of 15 cm near each termite trap during each termite collection between 0800 and 0900 hours.

Experimental Apparatus. To measure the CTMax, CTMin, and ULL, termites were tested in a custom-designed microprocessor-controlled incubator that either heated or cooled at a constant rate. Termite workers were confined individually in 5.5-ml plastic cups and placed into the incubator. A 5-ml container was filled with water and placed into the incubator chamber to prevent desiccation. This design maintained RH of >50% throughout the heating or cooling cycles as measured using a RH probe (Tri-Sense Meter; Cole Parmer, Niles, IL). The conventional 1°C/min rate of temperature change for thermal studies on terrestrial animals (Hutchinson 1961) was used in this study. Temperatures were programmed to either increase or decrease at 1°C/min, and knockdown was observed through the Plexiglas top of the incubator. Linearity and uniformity of heating and cooling was ±1.0% within the incubator. Knockdown was observed visually, and the temperature at which knockdown occurred was recorded for each termite.

Procedure for Determining CTMax, CTMin, and ULL. To select uninjured healthy mature worker foragers, each field collection was placed into a plastic container that was tilted 45°. From each colony, the first 20 workers that crawled up the side of the container were used in this study. To relate termite body mass with CTs and ULLs, workers of each species were weighed individually in microfuge tubes using an elec-

Table 1. Means of critical thermal maxima (CTMax), critical thermal minima (CTMin), and upper lethal limit for *C. formosanus* and *R. flavipes* workers ($n = 10$ for each parameter) collected monthly from field traps during a 1-yr period

Test date	*C. formosanus*				*R. flavipes*			
	Colony	CTMax (°C ± SE)	CTMin (°C ± SE)	ULL (°C ± SE)	Colony	CTMax (°C ± SE)	CTMin (°C ± SE)	ULL (°C ± SE)
10 Sept 2001	I	45.6 ± 0.3a	8.5 ± 0.2a	49.6 ± 0.2a	A	44.5 ± 0.3a	4.4 ± 0.3a	47.5 ± 0.3a
	II	45.8 ± 0.4a	8.5 ± 0.3a	49.8 ± 0.2a	B	44.0 ± 0.1b	4.2 ± 0.1a	47.6 ± 0.3a
	III	45.9 ± 0.2a	8.5 ± 0.1a	49.7 ± 0.2a	C	44.8 ± 0.3a	4.5 ± 0.1a	47.5 ± 0.2a
	IV	45.9 ± 0.2a	8.4 ± 0.1a	49.7 ± 0.3a	D	44.5 ± 0.2a	4.4 ± 0.1a	47.6 ± 0.1a
					E	44.8 ± 0.3a	4.4 ± 0.3a	47.5 ± 0.3a
12 Oct	II	45.7 ± 0.3a	8.0 ± 0.3a	49.5 ± 0.3a	A	44.7 ± 0.2a	4.8 ± 0.3a	47.4 ± 0.3a
	III	45.8 ± 0.2a	8.1 ± 0.2a	49.6 ± 0.2a	B	44.9 ± 0.2a	4.0 ± 0.1a	47.3 ± 0.2a
	IV	45.8 ± 0.1a	8.2 ± 0.1a	49.6 ± 0.1a	C	44.8 ± 0.3a	4.8 ± 0.2a	47.5 ± 0.2a
					E	44.7 ± 0.3a	4.1 ± 0.1a	47.4 ± 0.1a
16 Nov	II	45.7 ± 0.3a	8.0 ± 0.1a	49.5 ± 0.3a	A	44.6 ± 0.2a	3.3 ± 0.2a	47.3 ± 0.3a
	III	45.8 ± 0.2a	8.1 ± 0.2a	49.7 ± 0.2a	B	43.9 ± 0.3b	3.2 ± 0.3a	47.4 ± 0.2a
					C	44.6 ± 0.1a	3.3 ± 0.1a	47.3 ± 0.1a
					E	44.4 ± 0.2a	3.2 ± 0.2a	47.4 ± 0.1a
18 Dec	III	45.4 ± 0.3	7.3 ± 0.1	49.4 ± 0.3	A	43.9 ± 0.3a	2.9 ± 0.2a	47.2 ± 0.2a
					B	44.0 ± 0.2a	2.0 ± 0.1a	47.1 ± 0.3a
17 Jan 2002	III	45.0 ± 0.2	7.3 ± 0.1	49.5 ± 0.2	A	43.8 ± 0.3a	2.8 ± 0.2a	47.2 ± 0.1a
					D	43.5 ± 0.2a	2.6 ± 0.3a	47.1 ± 0.3a
					E	43.8 ± 0.1a	2.7 ± 0.1a	47.2 ± 0.2a
15 Feb	III	45.3 ± 0.3	7.2 ± 0.1	49.1 ± 0.2	A	43.6 ± 0.3a	1.5 ± 0.2a	47.0 ± 0.2a
					C	43.5 ± 0.2a	1.8 ± 0.1a	46.9 ± 0.4a
					D	43.7 ± 0.1a	1.5 ± 0.3a	47.0 ± 0.1a
14 Mar	III	45.3 ± 0.2	7.8 ± 0.3	49.2 ± 0.2	A	43.8 ± 0.3a	3.6 ± 0.2a	47.5 ± 0.2a
					D	44.0 ± 0.3a	3.2 ± 0.1b	47.3 ± 0.3a
13 April	II	44.8 ± 0.2a	8.2 ± 0.2a	49.3 ± 0.3a	A	44.3 ± 0.2a	3.9 ± 0.2a	47.1 ± 0.2a
	III	45.3 ± 0.2b	8.3 ± 0.1a	49.3 ± 0.1a	D	44.2 ± 0.3a	3.8 ± 0.3a	47.3 ± 0.3a
	IV	45.2 ± 0.1b	8.3 ± 0.3a	49.2 ± 0.2a	E	44.3 ± 0.1a	3.9 ± 0.1a	47.5 ± 0.1a
10 May	III	45.7 ± 0.1a	8.5 ± 0.2a	49.1 ± 0.2a	A	44.6 ± 0.2a	4.1 ± 0.3a	47.8 ± 0.2a
	IV	45.6 ± 0.2a	8.5 ± 0.3a	49.3 ± 0.3a	C	44.5 ± 0.3a	4.0 ± 0.3a	47.8 ± 0.1a
16 June	III	45.6 ± 0.1	8.7 ± 0.2	49.6 ± 0.2	A	44.1 ± 0.2a	4.7 ± 0.2a	47.8 ± 0.2a
					C	44.0 ± 0.3a	4.8 ± 0.3a	47.9 ± 0.3a
17 July	III	45.6 ± 0.2	9.0 ± 0.2	49.9 ± 0.2	A	44.2 ± 0.4a	4.8 ± 0.2a	48.2 ± 0.2a
					C	44.8 ± 0.2b	4.2 ± 0.1b	48.1 ± 0.3a
15 August	III	45.9 ± 0.1	9.0 ± 0.2	50.4 ± 0.2	A	44.9 ± 0.3a	4.8 ± 0.2a	48.3 ± 0.2a
					C	44.8 ± 0.2a	4.9 ± 0.1a	48.3 ± 0.1a

Means within columns and months followed by the same letter are not significantly different ($P = 0.5$).

tronic analytical balance (0.01 mg sensitivity, M-220D; Denver Instrument, Arvada, CO) immediately before their use. A minimum of 10 workers from each colony were used to determine each thermal tolerance measure.

In this study, CTMax and CTMin were defined as the high or low temperatures, respectively, at which a termite was knocked down and was unable to move one body length when probed, but that recovered fully within 15 min after removal from the incubator. The ULL was defined as the high temperature at which all body movements ceased and no recovery occurred within 1 h after removal (Sponsler and Appel 1991).

Statistical Analyses. All tests were performed using Statistix7 software (Analytical Software 2000). Data were analyzed for differences in the monthly CTMax, CTMin, and ULL values among colonies of each species, using one-way analysis of variance (ANOVA). The consecutive 12-mo data from two colonies, one of each species, were tested for seasonal variation and relation to corresponding soil temperatures. First the data were subjected to two-way ANOVA with split-plot design and Tukey's mean separation tests, in which sources of variance were species, month, and the species by month interaction. Subsequently, data were subjected to simple linear regression analyses in which monthly means of the CTMax, CTMin, and ULL values of each species were plotted against corresponding soil temperature measurements at a depth of 15 cm (independent variable). Regression lines were compared using the Comparison of Regression Lines options (Analytical Software 2000) to determine if the slopes were similar between species. Correlation was used to test the degree of linear association between termite temperature tolerances and their body masses. The 0.05 probability level was used in all tests of significance.

Results and Discussion

Colony Effect on Temperature Sensitivities. Critical thermal and ULL values of *C. formosanus* and *R. flavipes* colonies are presented in Table 1. ANOVAs did not reveal any conclusive effect of colonies on CTMax, CTMin, and ULL.

First, colonies did not demonstrate consistent variation in temperature sensitivity for different months. There were 5 mo when we obtained more than two *C. formosanus* colonies. Analyses of CTMax, CTMin, and

ULL data resulted in only one significant difference, which occurred in April when colony II had a significant lower CTMax value (44.8 ± 0.2°C) than colonies III (45.3 ± 0.2°C) and IV (45.2 ± 0.1°C; $F = 6.9$; df = 2,29; $P < 0.05$). With *R. flavipes,* we obtained samples from more than two colonies each month. Analyses of CTMax, CTMin, and ULL data resulted in only 5 significant differences between colonies of 36 tests (≈14%). Significant differences of CTMax values were found between colony B and colonies A, C, D, and E in September ($F = 2.5$; df = 4,49; $P < 0.05$) and between colony B and colonies A, C, and E in November ($F = 4.31$; df = 3,39; $P < 0.05$), but not in October or December ($P > 0.05$). In July, colony C had significantly different CTMax ($F = 7.51$; df = 1,19; $P < 0.05$) and CTMin values ($F = 6.54$; df = 1,19; $P < 0.05$) compared with colony A, but not in the other 8 mo when they were both available. In March, colony D had a lower CTMin value (3.2 ± 0.1°C) than colony A (3.6 ± 0.2°C; $F = 6.8$; df = 1,19; $P < 0.05$), but the differences between these two colonies in the other 5 mo were not significant.

Second, colony variability in temperature sensitivity was not consistent for CTMax, CTMin, and ULL. For instance, *C. formosanus* colony II had a significantly lower CTMax value ($P < 0.05$), but similar CTMin and ULL values, compared with colonies III and IV (CTMin: $F = 0.78$; df = 2, 29; $P > 0.05$; ULL: $F = 0.69$, df = 2,29; $P > 0.05$) in April. *R. flavipes* colony B had a significantly lower CTMax ($P < 0.05$), but similar CTMin and ULL values ($P > 0.05$) compared with other colonies in September and November. *R. flavipes* colony D had a significantly lower CTMin value than colony A ($P < 0.05$), but similar values of CTMax and ULL ($P > 0.05$) when measured in March. Moreover, *R. flavipes* colony C showed a significantly greater CTMax ($P < 0.05$), but a significantly lower CTMin ($P < 0.05$), and a similar ULL ($P > 0.05$) compared with colony A in July.

Variation among subterranean termite colonies has been reported in behavioral, genetic, and insecticide susceptibility studies (Shelton and Grace 1996, Husseneder and Grace 2001, Osbrink et al. 2001, Thorne and Breisch 2001). To our knowledge, there is no report on variation of temperature tolerances between termite colonies. Using the harvester termite in Africa, *Hodotermes mossambicus* (Hagen), Mitchell et al. (1993) presented the only analysis of possible colony effects on critical thermal limits. Similar to what we found in this study, Mitchell et al. (1993) reported inconsistent colony temperature sensitivity. They observed that the workers from one colony had a greater CTMax than workers from another colony, whereas the larvae from the first colony had a lower CTMax than the second colony, but the differences were not significant. They hypothesized that differing physiological tolerances of colonies might be related to the age or maturity of the individuals, previous thermal and feeding history, or the extent of water reserves (Mitchell et al. 1993).

Because our objectives focused on determining seasonal changes of temperature tolerance and associating the seasonal fluctuation with habitat temperatures during a 1-yr period, an appropriate data set should consist of data from the same colonies for a consecutive 12-mo period. We were able to obtain consecutive 12-mo data from *C. formosanus* colony III and *R. flavipes* colony A. The temperature tolerances of these two colonies were similar to those of most other colonies from which we obtained data (Table 1). Therefore, data from *C. formosanus* colony III and *R. flavipes* colony A were used for further analyses.

Specific and Seasonal Variations of CTs and ULL. Monthly mean values of CTMax, ULL, and CTMin were significantly greater for *C. formosanus* than for *R. flavipes* (CTMax: $F = 221.08$; df = 1, 239; $P < 0.05$; critical value = 0.18; ULL: $F = 703.2$; df = 1, 239; $P < 0.05$; critical value = 0.15; CTMin: $F = 2745.3$; df = 1, 239; $P < 0.05$; critical value = 0.16; Table 1; Figs. 1 and 2). Monthly mean CTMax values (Fig. 1A) ranged between 45.0 and 45.9°C for *C. formosanus* and between 43.6 and 44.9°C for *R. flavipes.* Monthly mean ULL values (Fig. 1B) varied between 49.1 and 50.4°C for *C. formosanus* and between 47.0 and 48.3°C for *R. flavipes.* Monthly CTMin values (Fig. 2) had a greater range, from 7.2 to 9.0°C for *C. formosanus* and from 1.5 to 4.8°C for *R. flavipes*, respectively.

Both termite species have significant seasonal variations of CTMax (Fig. 1A; $F = 3.65$; df = 11, 239; $P < 0.05$; critical value = 0. 78), ULL (Fig. 1B; $F = 5.94$; df = 11, 239; $P < 0.01$; critical value = 0.66), and CTMin (Fig. 2, $F = 32.33$; df = 11, 239; $P < 0.01$; critical values = 0.66). No significant differences were found for the interaction of month on species for CTMax ($F = 0.87$; df = 11, 239; $P > 0.05$) and ULL ($F = 1.79$; df = 11, 239; $P > 0.05$). However, the interaction of month on species was significant for CTMin ($F = 3.63$; df = 11, 47; $P = 0.02$). In general, the lowest values of each of the three thermal parameters were obtained in February, except for the CTMax value of *C. formosanus,* which fell in January. The highest values of all parameters were in August.

Relationships Between CTs/ULL and Seasonal Habitat Temperatures. CTMax, CTMin, and ULL values were regressed on 15-cm depth soil temperatures. Regardless of the low descriptive values of the R^2, the fact that the coefficients of determination were significant indicates that ground temperatures within the range measured in this study had effects on both CTMax (*C. formosanus:* $R^2 = 0.04$; $F = 3.52$; df = 1, 119; $P < 0.05$; *R. flavipes:* $R^2 = 0.06$; $F = 7.12$; df = 1, 119; $P < 0.05$) and ULL (*C. formosanus:* $R^2 = 0.08$; $F = 10.14$; df = 1, 119; $P < 0.05$; *R. flavipes:* $R^2 = 0.24$; $F = 38.13$; df = 1, 119; $P < 0.05$). Further comparisons of the CTMax slopes indicated that both termite species responded to ground temperature variation in a similar manner ($F = 1.16$; df = 1, 236; $P > 0.05$). Comparison of the ULL slopes revealed a significant difference between species ($F = 5.21$; df = 1, 236; $P < 0.05$).

Greater R^2 values were obtained for CTMin of both species (*C. formosanus:* $R^2 = 0.54$; $F = 140.35$; df = 1, 119; $P < 0.05$; *R. flavipes:* $R^2 = 0.52$; $F = 127.81$; df = 1, 119; $P < 0.05$), indicating that habitat ground tem-

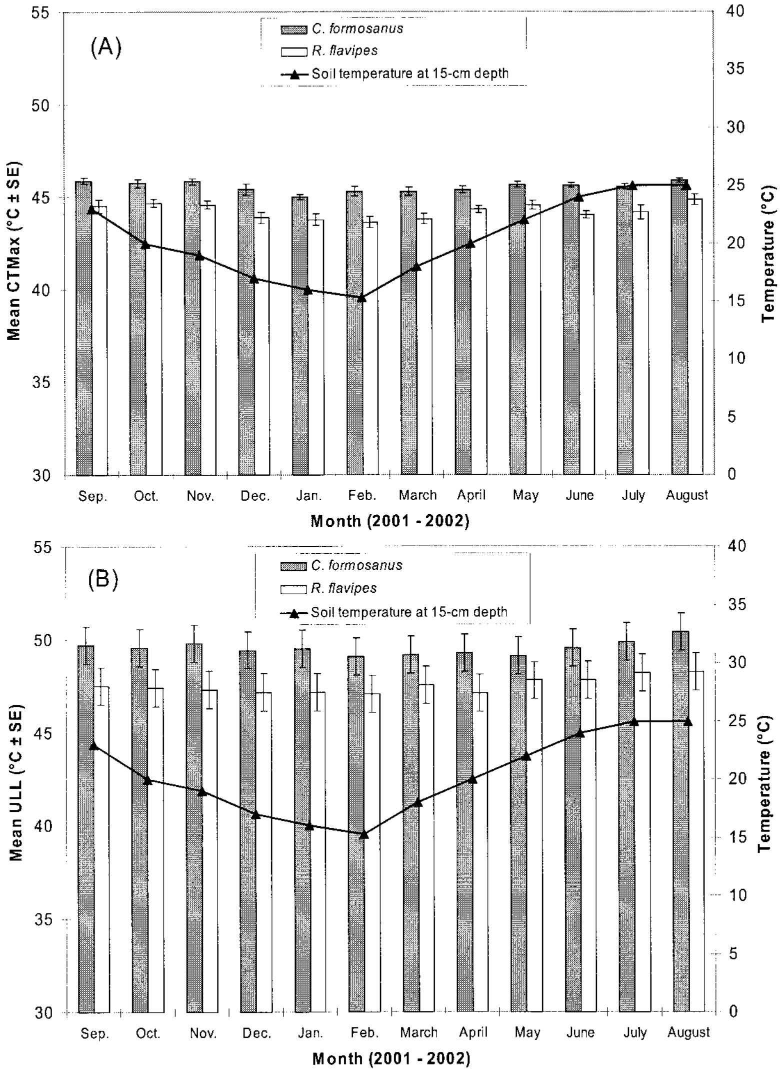

Fig. 1. Seasonal variation of the CTMax and ULL of the worker cast of *C. formosanus* (colony III) and *R. flavipes* (colony A). (A) CTMax. (B) ULL.

peratures within the range measured in this study were important in explaining the observed variation in CTMin values. Comparisons of equality of variances ($P < 0.05$), slopes ($P < 0.05$), and intercepts ($P < 0.05$) indicated that the linear regression lines were species specific (*C. formosanus*: $y = 0.18\ (\pm 0.02)x + 3.95\ (\pm 0.36)$; *R. flavipes*: $y = 0.23\ (\pm 0.03)x - 2.4\ (\pm 0.55)$, where y = CTMin and x = ground temperature at 15-cm depth). These results indicate that the two species responded to low temperatures in a similar manner but at different degrees of temperature sensitivity. The greater slope of *R. flavipes* indicates better acclimatization by this species to low temperatures than *C. formosanus.*

Correlation of CTs and ULL with Body Mass. There was no significant correlation between termite body mass and CTMax, ULL, or CTMin for either species (Pearson's r coefficient: CTMax: $P = 0.054$; ULL: $P = 0.64$; CTMin: $P = 0.20$; Fig. 3).

Our data clearly show that *C. formosanus* was more heat tolerant, and *R. flavipes* was more cold tolerant. Overall, the range of CTs was 7.2–45.9°C in *C. formosanus* and 1.5–44.9°C in *R. flavipes.* These results indicate that (1) the two termite species could have sympatric distributions from subtropical areas into temperature climates, where certain lower temperatures limit *C. formosanus* northward extension; (2) *C. formosanus* may survive better at higher temperatures; and (3) *R. flavipes* may survive better at colder temperatures. Our data could partially explain the current geographic distributions of the two termite species and the fact that *C. formosanus* is often found in aerial foraging situations and aerial colonies where ambient temperatures can be relatively high (Kofoid 1934). In

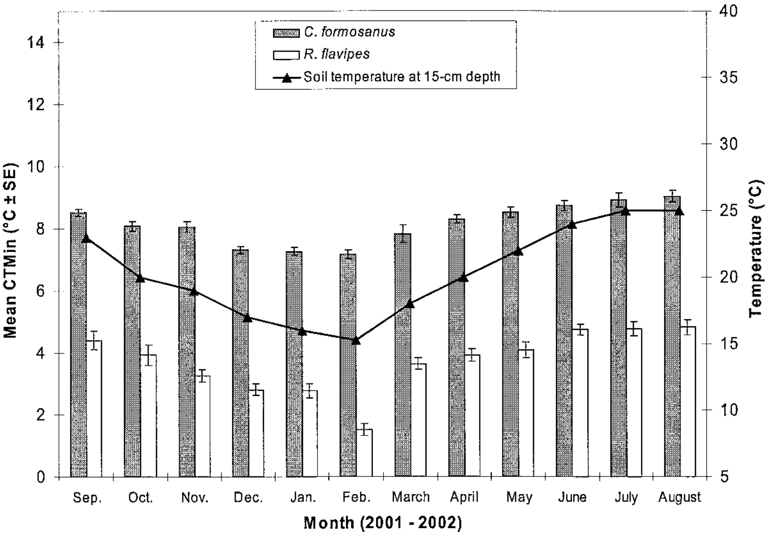

Fig. 2. Seasonal variation of the CTMin of the worker caste of *C. formosanus* (colony III) and *R. flavipes* (colony A).

contrast, *R. flavipes* in subtropical areas is generally restricted to subterranean habitats where the environment is cooler and has been found in locations with severe winters (Esenther 1969). Temperature values from foraging studies are lower than the CTMax (45.0–45.9°C for *C. formosanus;* 43.6–44.9°C for *R. flavipes*) and higher than the CTMin (7.2–9.0°C for *C. formosanus;* 1.5–4.9°C for *R. flavipes*) reported herein. Li (1991) reported a higher foraging limit of 10°C and a lower foraging limit of 40°C for *C. formosanus*. Strack and Myles (1997) found a major reduction in *R. flavipes* foraging activity at a temperature of 5°C. In our study, we noticed some lack of locomotor coordination 5–6°C before CTMax and CTMin in both species. This could be an indication that normal foraging ability was affected before the CT limits were reached. With a CTMin lower than the lower activity temperature, foragers would not be in danger of exposure to lethal temperatures. We attempted to measure the LLL, which is defined as the low temperature at which all body movements cease and no recovery occur within 1 h after removal (Sponsler and Appel 1991); however, all tested termite workers recovered. We continued to expose workers to decreasing temperatures until the temperature was 2, 4, 6, 8, and 10°C below the temperature at which termites ceased all body movement. Yet again, all termites recovered within 1 h after removal. As a result, we were not able to obtain LLL measurements in this study.

Our CTs and ULL values vary somewhat from those of previous studies. Sponsler and Appel (1991), using a less controlled thermal rate bioassay, generated greater CT values (CTMax: 46.3°C for *C. formosanu,* 45.4°C for *R. flavipes;* CTMin: 14.0°C for *C. formosanus,* 13.3°C for *R. flavipes*), but lower ULLs (48.0°C for *C. formosanus,* 46.4°C for *R. flavipes*), than the range of values reported in this study. Some of the differences among studies in thermal tolerance limits possibly could be explained by differing thermal history, age, and other parameters of individual workers (Mitchell et al. 1993), and most importantly, by different bioassay methods and observation techniques (Scheffrahn et al. 1997, Woodrow and Grace 1998). Woodrow and Grace (1998), for example, generated an ULL value of 42°C using a preset temperature method and an ULL value of 47.9°C using a thermal rate bioassay protocol. Researchers should be careful to report as much information on the thermal history, geographic location, colony conditions, and other parameters of individual termites as possible. Researchers also should be careful when drawing conclusions and interpreting results of studies involving quantification of temperature tolerance in termites because the results can be affected by when or where they are collected.

Our data provide the first empirical evidence that the CTs and ULL values in *C. formosanus* and *R. flavipes* fluctuate seasonally through a year. Seasonal maxima of CTs and ULLs occurred in the summer, and seasonal minima occurred in the winter. Regression analyses clearly indicate that termites adjusted their thermal tolerance capabilities in accordance with fluctuating seasonal habitat temperatures. The direction of the influence of acclimatization temperature is similar for CTMax, CTMin, and ULL, suggesting that both species have an ability to adapt (at least in part) to higher or lower temperatures as summer or winter approaches. Similarly, Mitchell et al. (1993) reported an increased value of CTMax with increasing acclimation temperatures and a decreased value of CTMin with decreasing acclimation temperatures for the harvester termite, *Hodotermes mossambicus* (Hagen), reared and maintained in the laboratory. Davis and Kamble (1994) noted decreased values of the LLL in field-collected *R. flavipes* in response to gradually cooling temperatures. However, the authors did not investigate the effect of cooling temperature on CTs,

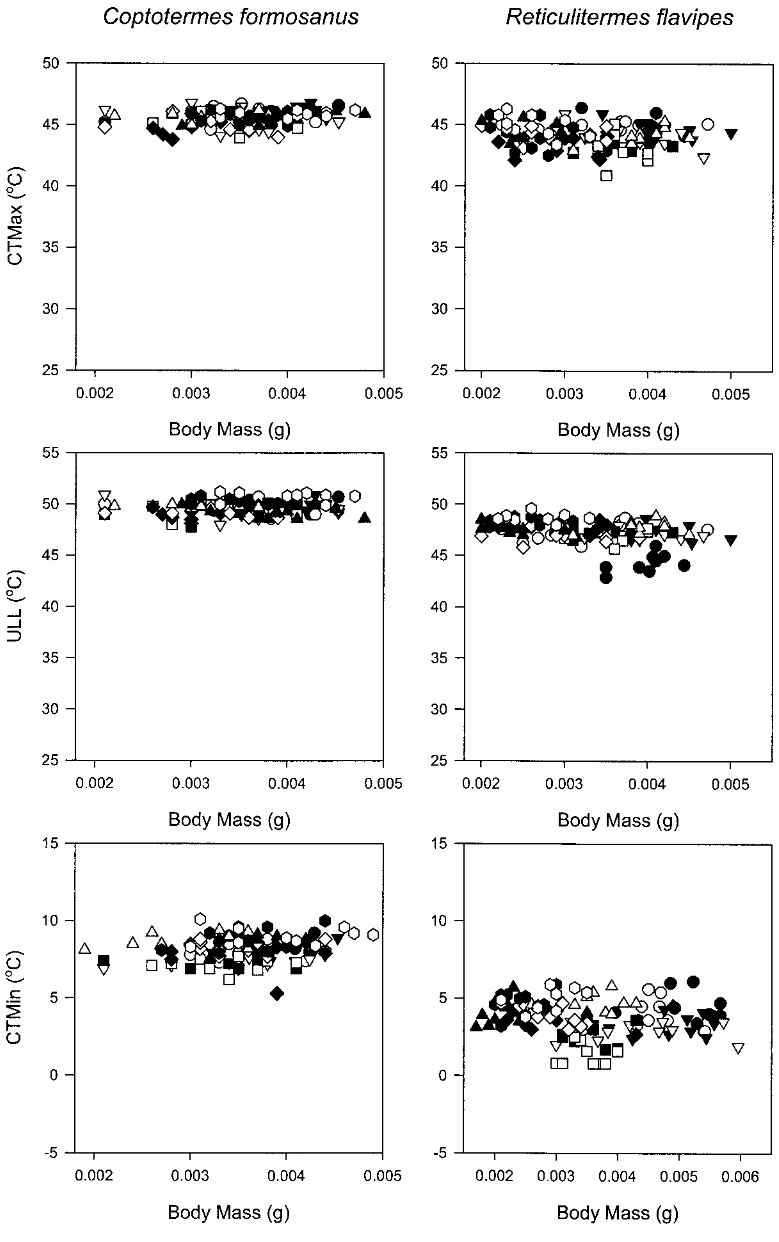

Fig. 3. Effects of termite body mass on CTMax, CTMin, and ULT of *C. formosanus* and *R. flavipes* workers. Months include the following: ●, September; ○, October; ▼, November; and ▽, December 2001; ■, January; □, February; ♦, March; ◇, April; ▲, May; △, June; ⬢, July; and ⬡ August 2002.

nor did they collect enough data for regression analysis.

These physiological thermal tolerance data probably do not indicate or suggest the exact distributions for *C. formosanus*, although the CTs represent the practical physiological limits of activity. The CTMin range of 7.2–9.1°C for *C. formosanus* in this study represents short-term, rapid exposure periods, rather than prolonged exposure that probably would occur in nature. Insect development and survival are functions of temperature and exposure time (Tamaki et al. 1980). Data on LLLs may help predict the distribution boundary. Sponsler and Appel (1991) reported an LLL of −2.9°C for *C. formosanus* and −3.0°C for *R. flavipes*. However, we were not able to obtain LLL values even when the temperature was lowered to −5.0°C, the lowest possible temperature obtainable using our experimental apparatus. Li (1991) reported that at −3.0°C, a 7-d exposure was required to kill 100% of *C. formosanus* workers. Li (1991) also reported that 9-, 14-, or 28-d exposures to −1, 1, or 4°C, respectively, were necessary to obtain 100% mortality. When exposed to 8°C, 90% mortality resulted after 34 d.

However, these physiological thermal limits may provide insight into the potential for introduction or establishment of *C. formosanus* into areas outside its current geographic range. The mean January 2002 temperature and the mean minimum at 10.16-cm depth reported by AWIS Weather Services (2002) (www.awis.com) were 10.6 and 8.3°C in Auburn, but 7.1 and 4.5°C in the northernmost part of AL. Soil temperature at a depth of 15 cm in January 2002 was 15.3°C at the collection site in this study, and it could be warmer deeper in the ground. The CTMin range of 7.18–9.06°C and the ability of *C. formosanus* to temperature acclimate suggest a high potential for further northward spread. Protected colonies and avoidance behaviors, in addition to their association with the more or less controlled temperatures in man-built structures, tend to enhance the chances of termite survival at low ambient temperatures below their thermal limits. Termite foragers may avoid exposure to critical temperatures by moving downward in the ground where temperature is more uniform (Strack and Myles 1997). For example, *R. flavipes* was found retreating in soil at a depth of >100 cm where winter freezing temperatures were not encountered (Esenther 1969, Husby 1980). Termites may also extend and build their foraging tunnels around heated structures, near sewer systems, or within tree stumps that provide habitable overwintering sites (Myles and Grace 1991, Davis and Potter 1996, Grace 1996). Thus we should be cautious when predicting potential distribution patterns of termites using only thermal tolerance limits, especially given new insulation technologies and central heating systems that could provide potential refuges against lethal temperatures. For example, *C. formosanus* is now established in northern Alabama where the mean winter temperature is ≥2°C, mean minimum temperature is ≥ −4°C, and a low temperature extreme of −15°C has been recorded (X. P. H., unpublished data).

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